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"Development and Validation of RP-HPLC Method for the Simultaneous Determination of Selumetinib in its Capsule Dosage Form"

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ABSTRACT

The primary goal of this study was to create a simple, precise, fast, and repeatable reverse phase high performance liquid chromatography (RP-HPLC) method for estimating Selumetinib in its capsule dose form. Chromatographic separation was performed on an Agilent Zorbax SB–C18 reversed phase column (2.1 mm x 100 mm, 3.5µm particle size, Agilent, USA) kept at room temperature with a 0.05M Phosphate buffer mobile phase. : Methanol mixed in the ratio of 60 : 40 v/v, was used at a flow rate of 1.0 ml/min, and the detection wavelength was set at 248.8 nm. The retention time of Selumetinib peak that was found to at 4.23 min. The developed method was linear in the range of 15 – 45 µg/ml with correlation coefficient of 0.9949 respectively. The %RSD of intraday and interday precision was found to be 0.458 and 0.069 respectively. The %RSD Values of intraday and interday precision were below two, that indicates the method which used was highly precise. The developed method was highly sensitive with LOD of 1.567 µg/ml and LOQ of 4.749µg/ml. Percentage assay of Selumetinib in capsule dosage formulation was found to be 99.563 %w/w. Thus the assay content of Selumetinib was determined and the mean % found for Selumetinib was in good agreement with lable claim. The proposed method was statistically validated for linearity and range, precision, accuracy, specificity and robustness. Thus the novel method of the Selumetinib was found to be feasible for the estimation of Selumetinib in it's capsule dosage form.

Key Words :- Selumetinib, RP-HPLC, Analytical method, Analytical Validation, Development, Validation, ICH guideline, Quantitative, Qualitatively.

INTRODUCTION

The chemical name of Selumetinib is 6-(4- Bromo-2-chloroanilino)-7-fluro-N-(2-Hydroxy ethoxy)-3-methylbezimidazole – 5-Carboxamide Figure 1. Mainly Selumetinib nowadays utilized for the treatment of the neurofibromatosis type-1(NF 1; a nervous system disorder that causes tumors to grow on nerves) in children 2yr of age & Older who have Plexiform neurofibromas (PN ; Soft tumors) that cannot be completely removed by Surgery.

A through literature survey of Selumetinib revealed that mainly 2-3 analytical methods had been reported for the estimation of Selumetinib. It was approved for medical use in the united states in April 2020. The U.S. Food and Drug Administration (FDA) considers it to be a first class medications.

Selumetinib is an official in U.S. pharmacopoeias. Selumetinib is sold under the brand name Koselugo, is a medication forthe treatment of children, two years of age and older, with neurofibromatosis Type-I (NF-I), a genetic disoder of the nervous system causing tumors to grow on nerves. This novel method gives correct peak shape, precise, simple, and quick use of smaller sample volumes with phosphate buffers and methanol as a mobile phase. So it is necessary to develop a simple, precise, accurate and validate RP-HPLC method for the quantitative as well as qualitative determination of Selumetinib drug. These all validation parameters stated by the International Conference on Harmonization [ICH] guidelines Q2 (R1). The chemical structure of Selumetinib is shown in Figure 1.

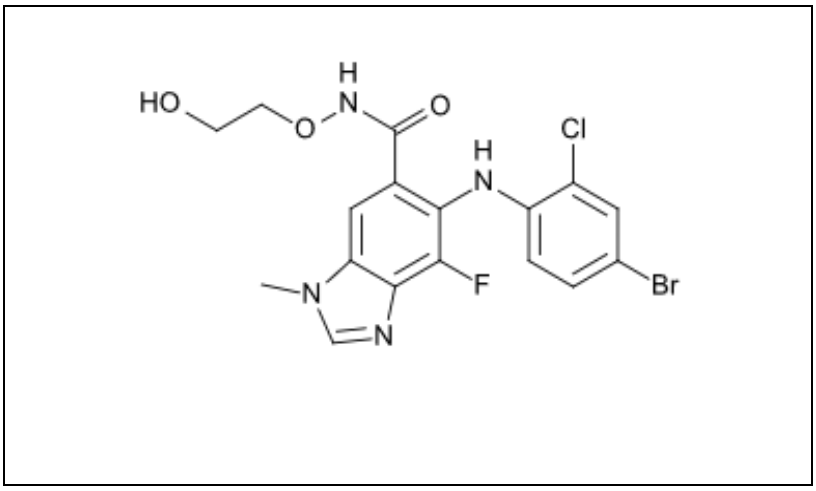

Figure 1 : Chemical Structure of Selumetinib

MATERIALS AND METHODS

Chemicals and Reagents: An analytically pure sample of Selumetinib standard was gifted by WOCKHARDT LIFE WINS, Bhimpore, Nani Daman Gujrat, India. All the chemical used in this method were high – grade purity and purchased from Merck Chemical Division Ltd., Mumbai, India. HPLC grade acetonitrile, water, methanol, ethanol, sodium perchlorate, hydrochloric acid were obtained from Merck Pharmaceutical Pvt. Ltd., Mumbai, India. Koselugo capsule containing Selumetinib with labeled amount 10mg as well as 25mg per capsule are manufactured by AstraZeneca Pharma Ltd., Mumbai, India.

Instrumentation and Conditions: The High Performance Liquid Chromatography analysis was carried out on a Reverse Phase High Performance Liquid Chromatography (RP-HPLC)(Shimadzu HPLC LC 2010), programmable variable wavelength Shimadzu 1800 UV- Vis detector and Column Agilent Zorbax SB – C18 reversed phase column (2.1 mm x 100mm, 3.5 µm particle size, Agilent, USA). In addition an electronic wt. balance (Shimadzu), digital pH meter (Thermometer), an ultrasonicator (Ultrasonic cleaner power sonic 420), UV-VIS Spectrophotometer (Shimadzu 1800) were utilized in this study.

Preparation of Mobile Phase: To prepare mobile phase HPLC grade 0.05M Phosphate buffer pH-5 and methanol were ratio 60 : 40 %v/v and was filtered through 0.45µm nylon filter and degassed by Sonication.

Preparation of Stock and Working Standard Solution: Accurately weighted quantity of Selumetinib 25 mg was transferred in 100ml volumetric flask. Dissolved and diluted up to mark with Methanol. This will give a stock solution having strength of 100µg/ml. Take 1ml from the Sesumetinib stock solution and transferred to 10mlvolumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Accurately weigh 6.8 gm of potassium di-hydrogen phosphate and dissolve in 1000ml of water (pH-4.8±0.02). Filter the buffer with 0.45µ nylon filter. This will give a solution of 0.05M Phosphate bufferpH-4.8.

Sample Preparation for Capsule: Weigh and powdered 20 Capsule. Take capsule powder equivalent to 25mg Selumetinib in to a 100ml volumetric flask. Add 60 ml methanol. Shake for 15 minutes and sonicate for 10 minutes. Make up volume with methanol. Filter this solution with Whatman filter paper no-1.

Appropriate volumes (15-35 µg/ml of Selumetinib) of this stock solution were then further diluted with ammonium acetate to 1ml to get the required concentrations of standard solutions at a concentration range of 15-35µg/ml. Accurately weighed 4gm of NaOH was transferred in100ml volumetric flask and diluted up to mark with methanol. From above solution take 10ml and transferred in 100ml volumetric flask and diluted up to the mark with methanol. Concentrated Hcl (0.86ml) was transferred in 100ml volumetric flask and dilute up to the mark with methanol.

Method Development: The objective of this experiment was to optimize the RP-HPLC method for the estimation of Selumetinib, based on the literature survey made.

In developing this method, a systematic study of effects of various parameters was under taken by varying one parameter at a time and controlling all other parameters.

A) Selection of Stationary phase
B) Selection of Mobile phase
C) Selection of wavelength

The following trials were carried out in order to optimize the RP-HPLC conditions for the estimation of Selumetinib in Capsule pharmaceutical dosage forms for further validation of the method conditions and *in vitro* dissolution samples analysis.

Table 1. List of Mobile Phase Trials for HPLC

Sr.No.	Mobile Phase	Mobile Phase Ratio	Result and Clue for Next Trial
1	HPLC Water : Methanol	50 :50 %v/v	No peak eluted
2	0.05M Phosphate buffer pH 4.8 : Acetonitrile	70 : 30 %v/v	Retention time of 3.36 min but peak fronting observed
3	0.05M Phosphate buffer pH 4.8 : Acetonitrile	80 : 20 %v/v	Retention time of 3.23 min but peak symmetry was not good
4	0.05M Phosphate buffer pH 5 : Acetonitrile	85 : 15 %v/v	Retention time 4.98 min peak fronting and broad peak shape
5	0.05M Phosphate buffer pH 5 : Methanol	60 : 40 %v/v	Retention time at 4.17 min with tailing factor below 2 and good peak symmetry

Method Optimization: Mobile phase optimization was initially carried out with Agilent Zorbax SB-C18 reversed-phase column (2.1mm× 100 mm, 3.5 µm, Agilent, USA) using HPLC Water : Methanol(50:50). Selumetinib peak was not eluted. In the next trial change in mobile phase 0.05M Phosphate buffer (pH-4.8) : Acetonitrile (70:30v/v) were tried on the same column, Selumetinib eluted with retention time of 3.36 min but peak and fronting was observed. In next trial, with same column, change in the mobile phase ratio of 0.05M Phosphate buffer (pH-4.8): Acetonitrile (80:20v/v). Selumetinib eluted at 3.50 min, fronting was observed, the peak symmetry was not good and baseline drift was observed. In another trail change in ratio 0.05M Phosphate buffer (pH-5) : Acetonitrile (85:15v/v) was used. The peak shape of Selumetinib was broad, and peak fronting was detected. In the next trial, the mobile phase was changed to 0.05M Phosphate buffer (pH-5): Methanol (60:40v/v), and the Selumetinib peak eluted at 4.23 min, with a tailing factor of less than 2, no fronting, and good peak symmetry. The wavelength was fixed at 248nm for quantitative analytical purposes, which gave superior reproducibility with minimal or no interference. The approach was validated in accordance with the ICH Guidelines. Selumetinib's peak purity index was found to be greater than 0.9996, indicating that the drug sample utilised in the assay was pure.

Validation of Analytical Methods: Once the chromatographic and experimental conditions were established, the technique was validated using the ICH Q2 (R1) criteria for specificity, accuracy, precision, system appropriateness study, robustness, LOD, and LOQ.

Specificity: The ability to assess the analyte definitively in the presence of components that might be present is referred to as specificity. Impurities, degradants, and matrix, for example, are typical examples. The effect of a wide range of excipients and other additives commonly found in Selumetinib formulations on optimum conditions determinations was examined.
To assess interference, the diluent, placebo, standard solution, and sample solution were each examined separately according to the procedure. There were no coeluting or interfering peaks where the drug peak eluted, implying that there were nocoeluting or interfering peaks.

This demonstrates that the analyte peak was pure and that excipients in the formulation had no effectt on the analyte. The peak purity indices of both the standardand sample peaks were determined to be greater than 0.9999, which agreed well with the previous findings. The lack of contaminants in the pure Selumetinib sample is also confirmed by the peak purity index (1.0000).

Linearity and range : Selumetinib was selected in the range of 15- 45g/ml. To acquire Selumetinib, pipette out 15, 20, 25, 30, 45 ml solutions from the Stock solution of Selumetinib (25g/mL) transfer to a 10 ml volumetric flask, and to

pup with mobile phase. The correlation coefficients for Selumetinib and Selumetinib calibration curves were found to be 0.9949 and 0.9949,respectively.

Precision :y=8327.9x-3955.2 for Selumetinib.
(A) Repeatability : Selumetinib (25g/ml) was determined using six measurements of the same Selumetinib (25g/ml) solution. Selumetinib was discovered to have a percent RSD of 0.648.
(B) Intraday Precision : Precision throughout the day.
(C) Interday Precision : Three times on the same day, a standard solution containing (20,25,30g/ml) of Selumetinib was evaluated, and the percent R.S.D. was computed.

Accuracy: 25µg/m drug solutions were taken in three different flask label A,B and C and Spiked 80%, 100%, 120% of standard solution in it and diluted upto l0ml. The Peak Area of each solution peak was measured at 248nm. The amount of Selumetinib was calculated at each level and % recoveries were computed.

LOD and LOQ :
For,
LOD = 3.3 * SD/slope of calibration curve For, LOQ = 10 * SD/slope of calibration curve Where, SD = Standard deviation of intercepts

Robustness: Change in parameter like flow rate, pH of mobile phase robustness were calcu1ated as per given table.
1. Flow rate of mobile phase was changed: (A0.2ml/min)0.5ml/minand1.2ml/ruin.
2. pH of Mobile phase

System suitability Test : It is an integral part of chromatographic method. The system suitability tests are used to ensure that the system's resolution and reproducibility are sufficient for the analysis. The principle behind system suitability tests is that the equipment, electronics, analytical operations, and samples all work together to for man integrated system that can be evaluated as a whole. System appropriateness testing ensures that the approach will deliver accurate and exact data for it's intended application.

For the system suitability test, the following values were observed:
1. Resolution (Rs): Are solution of 11.254 was observed.
2. Column efficiency (N): For Selumetinib, the number of plates observed was 4612.
3. Tailing factor for Selumetinib: 1.364 was observed as Symmetry factor(S).

The developed method was used to examine acommercially available formulation:
Selumetinib 25(g/ml) Sample Stock Solution: 10mL of sample volume (equal to 25mg Selumetinib) was transferred to a 100mL volumetric flask, 50mL Mobile phase was added, and the flask was shaken for 15 minutes before making up the volume with Mobile phase. Whatmann filter paper was used to filter the solution.

RESULT AND DISCUSSION

Specificity:

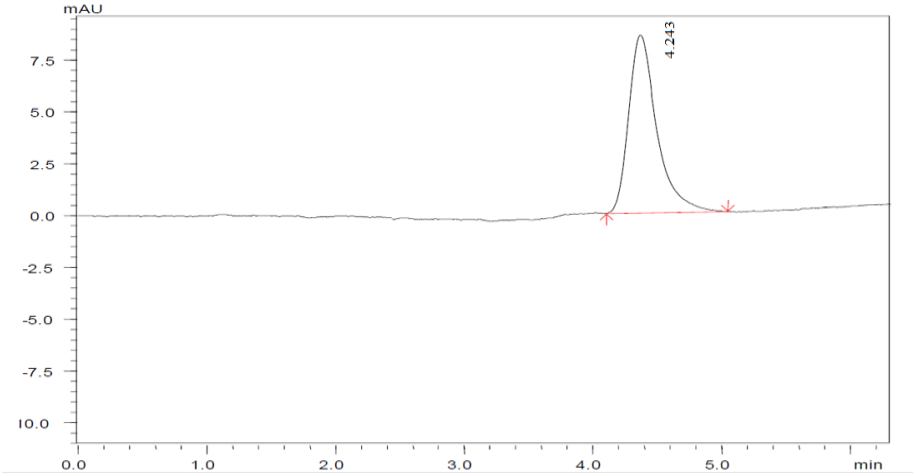

Figure 2 Standard for Specificity of Selumetinib

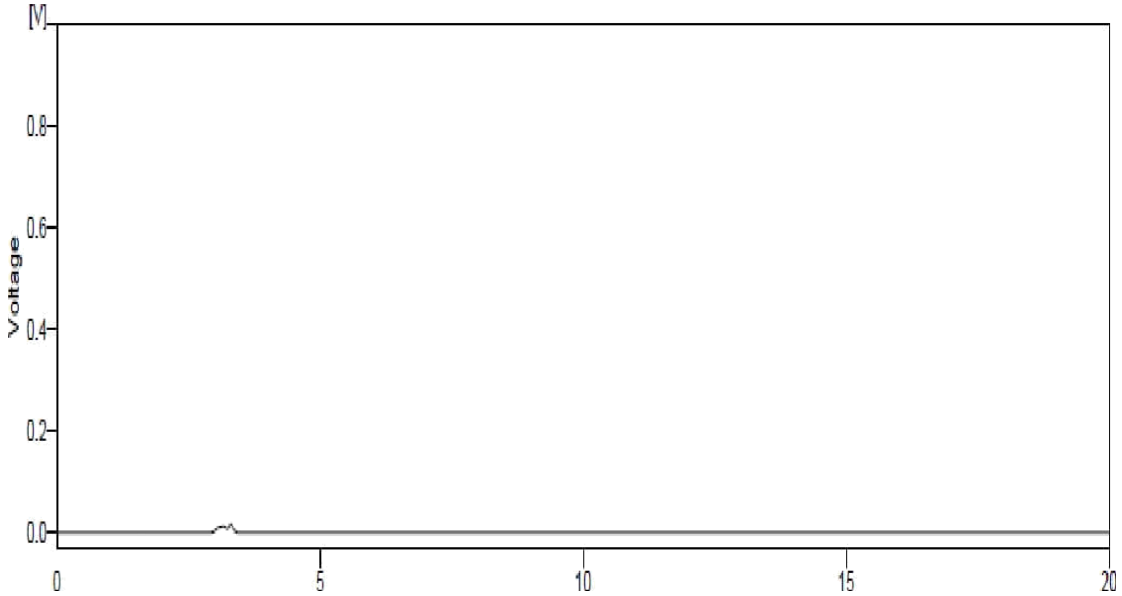

Figure 3 Blank for specificity

Linearity and Range:

Table 2 Linearity and Range of Selumetinib

Sr. No	Concentration (µg/mL)	Peak Area
1	15	109793.366
2	20	156711.312
3	25	209654.341
4	30	258963.362
5	45	335417.321

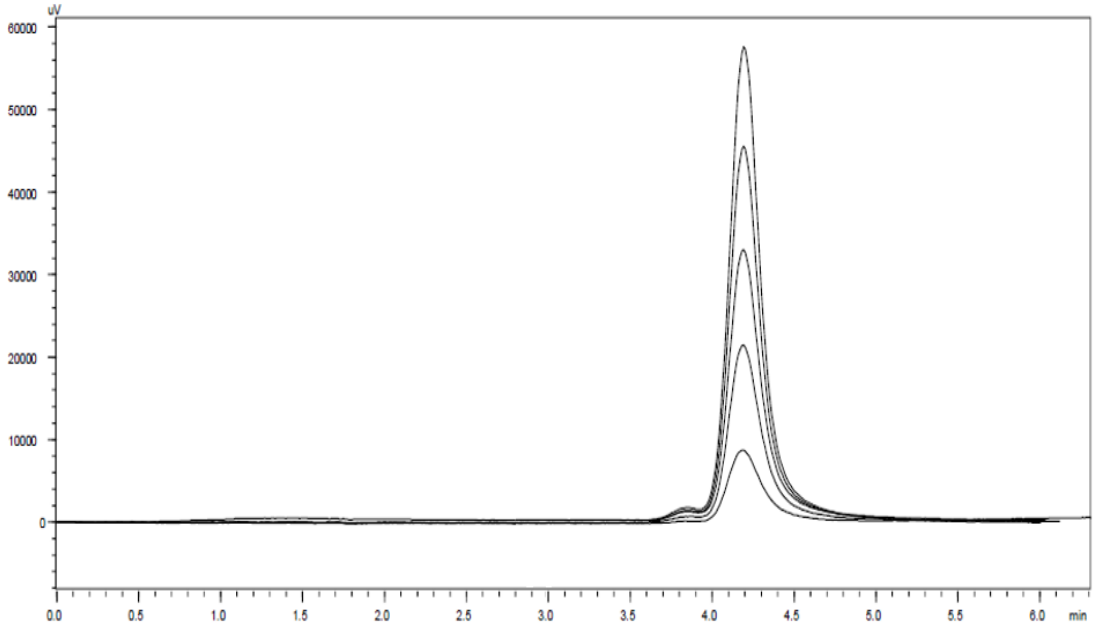

Figure 4 Overlay chromatogram for linearity

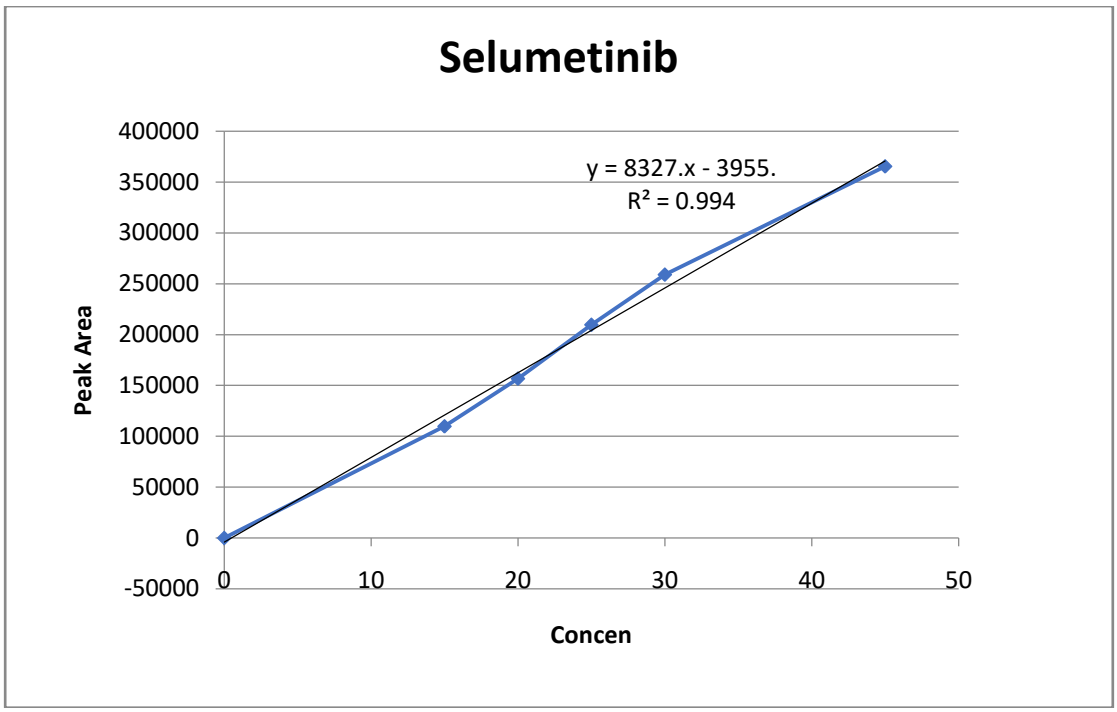

Figure 5 Calibration Curve of Selumetinib (15-45 µg/ml)

Precision:

(A) Repeatability

Selumetinib (25 µg/ml) based on six measurements of same solution of Selumetinib (25 µg/ml). The % RSD Selumetinib and was found to be 0.648 respectively.

Table 3 Repeatability of Selumetinib

Selumetinib				
Sr No.	**Conc. (µg/ml)**	**PeakArea**	**Mean ± S.D(n=6)**	**% R.S.D**
1.	25	4365.895	4365.436±28.226	0.648
		4374.541		
		4310.841		
		4392.068		
		4370.365		
		4378.954		

(B) Intraday precision

Standard solution containing (20, 25, 30µg /ml) of Selumetinib were analyzed three times on the same day and % R.S.D was calculated.

Table 4 Intraday precision data for estimation of Selumetinib

	Selumetinib		
Sr. No.	Concentration (µg/ml)	PeakArea Mean ± S.D. (n=3)	% R.S.D
1	20	443881	0.458
2	25	442863	
3	30	446789	

(C) Interday precision

Table 5 Interday Precision data for estimation of Selumetinib

Sr. No.	Concentration (µg/ml)	Peak Area Mean ±S.D. (n=3)	% R.S.D
1	20	443625	0.069
2	25	444242	
3	30	443890	

Accuracy :25µg/ml drug solutions were taken in three different flask label A,B and C and Spiked 80%, 100%, 120% of standard solution in it and diluted upto l0ml. The Peak Area of each solution peak was measured at 248nm. The amount of Selumetinib was calculated at each level and % recoveries were computed.

Table 1.6 Accuracy of Selumetinib

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80%	25	20	20.2415466	98.745	99.812 ± 1.028
2		25	20	20.2156354	100.728	
3		25	20	20.3245385	100.195	
4	100%	25	25	25.3162040	99.047	99.616 ± 0.541
5		25	25	24.4352777	100.144	
6		25	25	24.3164769	99.649	
7	120%	25	30	29.0026549	100.006	99.632 ± 0.564
8		25	30	30.3214826	99.184	
9		25	30	29.9695254	99.701	

LOD and LOQ:

Table 7 LOD for Selumetinib

Selumetinib
LOD = 3.3 x (SD / Slope) = 3.3 x (3955.2/8327.9) = 1.567µg/ml

Table 8 LOQ for Selumetinib

Selumetinib
LOD = 3.3 x (SD / Slope) = 10 x (3955.2/8327.9) = 4.749µg/ml

Robustness:

Table 9 Robustness for Selumetinib

Sr. no.	Peak Areaat Flow rate(-0.2ml/min)	Peak Area It Flow rate (+ 0.2 ml/min)	Peak Area atpH (- 0.2)	Peak Area atpH (+ 0.2	Peak Area atMobile phase(-2)	PeakAreaat Mobile phase(+2)
1	4610.109	4398.910	4569.112	4151.521	4489.981	4218.136
2	4546.564	4368.251	4501.951	4194.546	4481.281	4273.321
3	4583.142	4436.214	4632.362	4212.174	4446.161	4304.320
RSD	0.693	0.773	1.277	0.745	0.518	1.023

System Suitability Test:

Table 10 System suitability parameters of optimized chromatogram Based

1

Drug	Retention time(min)	Asymmetry	Theoretical Plate	Resolution
Selumetinib	4.230	1.364	4612	11.254

Analysis of Marketed Formulation:

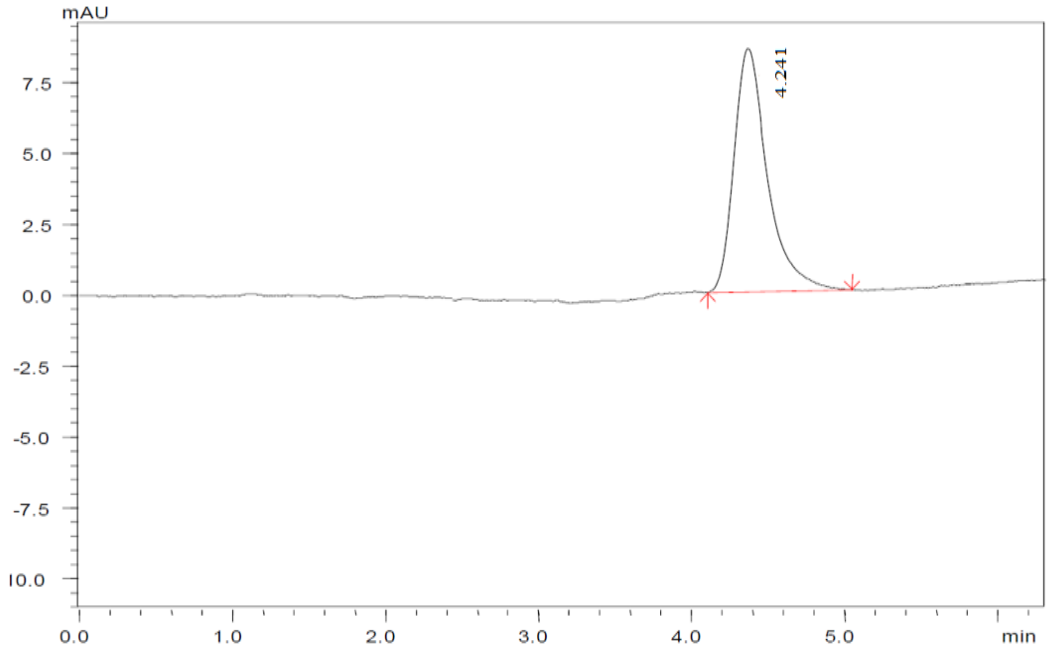

Figure 6 Selumetinib assay sample

Table 11Analysis of marketed formulation by developed method

	Selumetinib
Assay (% of label claim*) Mean ± S. D	99.563 ±1.066

SUMMARY AND CONCLUSION

Method Development:

Finally, after doing appropriate trials, these are the optimum conditions.
Phase of mobility: Methanol (60:40v/v) : 0.05M Phosphate buffer (pH-5)
Flow Rate: 1.0ml/min
Type of Evalution: Isocratic
Wavelength of Detector: 248nm
Temperature of the column: Ambient
Injection volume: 10uL
Running time: 8-minute
Peak was eluted at 4.23 minutes under the optimum conditions, with good peak symmetry and acceptable results for system suitability parameters.
Validation of the Method:

Specificity

Spiking solutions of routinely used excipients was utilized to test the method's specificity. Peak purity tests were also performed to demonstrate that the analyte chromatographic peak is not attributable to more than one component because impurities are not available through purity index data and there was no interference from impurities with the analyte peak. This demonstrates that the analyte peak was pure and that excipients in the formulation had no effect on the analyte.

Linearity

By building a graph between concentrations vs peak regions, linearity studies were done at 15 - 45g/ml of Selumetinib

Standard drug solution. The percent RSD of peak areas was calculated. The correlation coefficient was calculated, and the data was found to be with In the 0.9949 approval requirements.

The result was found to be within limit so the method is linear over the concentration range of 15-45 µg/mL of Selumetinib.

Precision

Precision of related substances were verified by repeatability. Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration (25µg/ml of Selumetinib) standard deviation and relative standard deviation were reported for precision.

- %RSD of area response of Selumetinib was 0.648 for Repeatability and 0.317 for method precision.
- %RSD of retention times of Selumetinib was 0.458 for Intraday precision and 0.061 for method precision.
-%RSD of retention times of Selumetinib was 0.069 for Interday precision and 0.061 for method precision.
-%RSD should be NMT 2 for areas and NMT 1 for retention times. It indicates that the method is precise.

Accuracy

Accuracy of the proposed method was ascertained by performing recovery studies by standard addition method by spiking the known quantities of standard at 80%, 100%, 120% to the drug product solution comprising of 25µg/mL. The % RSD and the % Recovery were within the acceptable limits of 98-102 in all cases.

The recovery of Selumetinib was found to be 99.812-99.632 %, which indicates a good accuracy of the method to that of the labelclaim.

LOD &LOQ

The quantification limit of individual analytical procedure is the lowest amount of analyte in the sample that can be determined quantitatively. LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve.

- LOD for Selumetinib was found to be 1.567µg/ml, indicating high sensitivity of the method.
- LOQ for Selumetinib was found to be 4.749 µg/ml µg/mL, indicating high sensitivity of the method.

Robustness

As part of the robustness, deliberate changes in the flow rate and wavelength were made to evaluate the impact on the method.

- The obtained results indicated that the minor changes in the flow rate and wave length did not affect the actual conditions.
- The Final value should be within 2% of the initial value.

Assay

The assay of Selumetinib Capsule was performed by comparing the areas of standard Selumetinib and Capsule sample,

- The percentage assay for Selumetinib was found to be 99.563 ± 1.066%.
- Assay was performed by the data was found suitable and within the acceptance range of 100 ± 2%.

Observed values for system suitability test:

Resolution (Rs): Resolution was observed 11.254.

Column efficiency (N): Number of plates observed for Selumetinib was 4612, respectively.

Symmetry factor (S): Tailing factor observed for Selumetinib1.364 respectively.

CONCLUSION

Finally, it can be concluded that the proposed RP-HPLC method was validated fully as per the International Conference on Harmonization (ICH) Guidelines, and found to be applicable for routine quality control analysis for the estimation of Selumetinib. The results of linearity, precision, accuracy and specificity proved to be within the limits. The method provides selective quantification of Selumetinib without interference from blank, placebo and degradants. The proposed

method is sensitive, reproducible, reliable, rapid, economical, and specific and LC-MS compatible, can be used for the estimation of Selumetinib.

REFERENCES

[1]. Sharma BK, Instrumental methods of chemical analysis, 7th Ed, Goel Publishing House, Meerut, 2000, 1-2.
[2]. David CL, Pharmaceutical analysis, 6th Ed, Black well publishing, London, 1994, 2-4.
[3]. Chatten LG, Pharmaceutical chemistry, Vol. II, Marcel Dekker Inc, New York, 1996, 23-25.
[4]. Beckett AH, Stenlake JB, Practical pharmaceutical chemistry, Vol. II, CBS publisher and distributors, New Delhi, 1986, 13-17.
[5]. Ashish C., Bharti M. and Priyanka C. "Analytical method development and validation: A concise review". *J Anal Bioanal Tech*.**2015** February Pg: 233-238
[6]. Rajani P., Ma Muthukumaran and B Krishnamoorthy. "A review on analytical method development and validation of pharmaceutical technology". *Pharmatutor*. ART-1671
[7]. Massom Raza S., Zei A Alothman and Nafisur R. "Analytical techniques in pharmaceutical analysis: A review". *Arabian J Chem*. **2013** April; 1-12
[8]. Ms Pallavi Nemgonda Patil "Hplc Method Development - A Review" *Journal of Pharmaceutical Research & Education*, **2017**, 1(2), 243-260
[9]. Yuri K. and Rosario L. "HPLC for pharmaceutical scientists" *Wiley interesciencepublication.* **2007**. 11-20
[10]. Bharat D., Rakesh G. and Dilip A. "A Review On: Development and Validation of HPLC in Pharmaceutical Dosage Form" *Asian Journal of Pharmaceutical Research and Development.* **2020**, 8(4), 110-121.
[11]. Thompson M, Ellison SLR and Wood R. "Harmonized guidelines for single laboratory validation of method of analysis-IUPAC" *technical report. Pure Appl Chem.* **2002**.835-855
[12]. Shabir G A, Lough W J, Arian S A and Bradshaw T K. "Evaluation and application of best practice in analytical method validation". *J Chromatogr*. **2007**, 311-330.
[13]. Dr S Ravi Sankar Text book of Pharmaceutical analysis. 4th edition. Rx Publications. 2012. 18.1-18-15
[14]. FDA, "Guidance for Industry; Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration," Rockville, US Departmentof Health and Human Services, 2000.
[15]. Connors K.A A Textbook of Pharmaceutical Analysis. 3rd edition. New Delhi; Wiley-Interscience Publication: 2002. 173-214
[16]. ICH Guideline Q7 (Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients) November 2000.
[17]. Jack Cazes Analytical instrumentation handbook Marcel Dekker Publishers,2005. 996-1012.
[18]. Sharma A and Saini S; "Process Validation of Solid Dosage Form: A Review." International Journal of Research in Pharmacy and Science, **2013**; 3 (2): 12- 30
[19]. Quantity assurance pharmaceutical (A Compendium of guidelines and related materials). 1997. Vol 1. WHO, Geneva, 119-124
[20]. Kaur H, Singh G and Seth N; "Pharmaceutical Process Validation: A Review." **2013**; 3 (4): 189-194.
[21]. Maulikkumar D Vaja, Bhoomi D Patel and Krina "A Review on process validation of solid dosage form" *Journal of Drug Delivery & Therapeutics* **2021**, 11(4), 157-160.
[22]. 22."Selumetinib"June,2020,https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-selumetinib-neurofibromatosis-type-1-symptomatic-inoperable-plexiform-neurofibromas
[23]. Paul S., Christopher B. and Meng Chen "Determination of selumetinib, N- esmethyl selumetinib and selumetinib amide in human biological samples by LC–MS/MS". *Bioanalysis* **2016**, 1-18.
[24]. Ramakrishna R., Theodore S Brus and Chineta, "Novel LC–MS/MS method for the determination of selumetinib (AZD6244) in whole blood collected with volumetric absorptive microsampling" *Bioanalysis* **2020** 57, 1-10.
[25]. Yanping Liu, Hua Liu and Zhewei Xia "Simultaneous and Rapid Determination of Six Tyrosine Kinase Inhibitors in Patients with Non-Small Cell Lung Cancer Using HPLC-MS/MS**"** *International Journal of Analytical Chemistry* **2021,** 1-9.
[26]. Murugan S, Sunil kumar V and Vineela Ruth Madhuri P,. "A New HPLC–UV Validated Method for Therapeutic Drug Monitoring of Tyrosine Kinase Inhibitors in Leukemic Patients" *Journal of Chromatographic Science.* **2011**, 49, pp 753-757.
[27]. Syeda K., Rahul Reddy.C and M.Kanaka Durga "Stimulation of ultraviolet-induced apoptosis of human ¢broblast UVr-1 cells by tyrosine kinase inhibitors" *Federation of European Biochemical Societies* **1999,** 173-176.
[28]. Sandra Roche and Martin Clynes "The Quantitative Analysis Of Tyrosine Kinase Inhibitor Levels In Biological Samples By LC-MS". *National Institute of Cellular Biotechnology*..**2008 ,**1.

[29]. Lutz G., Axel H. and Stephan Klaus Metzelder, "Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma". *Clinica Chimica Acta* **2012**, 14(3), 143–149.
[30]. Pirro E, De Francia S, De Martino F and Fava. "A new HPLC-UV validated method for therapeutic drug monitoring of tyrosine kinase inhibitors in leukemic patients.". *Journal Of Chromatographic Science* **2021**, 49 (10) 753-757.
[31]. Nasr Y. Khalil, Ibrahim A. Darwish and Munif F,. "ICH Guidelines-compliant HPLC-UV Method for Pharmaceutical Quality Control and Therapeutic Drug Monitoring of the Multi-targeted Tyrosine Kinase Inhibitor Pazopanib". *S. Afr. J. Chem*, **2017**, 70, 60-66.
[32]. Kohei T., Kenta F. and Kotaro Y. "Facile And Sensitive Hplc-Uv Method For Determination Of Nintedanib In Rat Plasma". *Int J Pharm Pharm Sci* **2018**, 10(6), 133 – 137.
[33]. Álvaro C., Silvia J. and Beatriz C. "Validation and Comparison of Two Analytical Methods for Imatinib Therapeutic Drug Monitoring". *Chromatographia* **2021,** 1-8.
[34]. Santosh V. and Barkha G. "Development and Validation of UV Spectroscopic Method for Estimation of Baricitinib" *Journal of Drug Delivery & Therapeutics* **2019**, 9(4), 488-491.
[35]. Vanesa E. and Juan J. "Development and Validation of an HPLC-UV Method for Pazopanib Quantification in Human Plasma and Application to Patients With Cancer in Routine Clinical Practice." *Ther Drug Monit.* **2015,** 37, 172-179.
[36]. Benoit B., Carole S. and Anne S. "Development and validation of an HPLC-UV-visible `method for sunitinib quantification in human plasma". *Clinica Chimica Acta* **2009,** 5,(6) 134-139
[37]. P. Ravi , A. Bhavani , M. M. Eswarudu and M. Nithya "Analytical Methods For Determination Of Different Members Of FDA approved Tyrosine Kinase Inhibitors Like Dasatinib, Lapatinib, Imatinib, Sorafenib, Nintedanib, Sunitinib And Pazopanib: A Review" *J. Pharm. Sci. & Res.* **2021**, 13(6), 313-318.
[38]. Hadir M., Aliyah A. and Nourah "Development and validation of UPLC–MS/MS method for thesimultaneous quantification of anaplastic lymphoma kinaseinhibitors, alectinib, ceritinib, and crizotinib in Wistar rat plasma with application to bromelain-induced pharmacokinetic interaction" *Journal of Pharmaceutical and Biomedical Analysis* **2021**, 1-9.
[39]. M Rajavardhan , Kirtimaya M., P.K.Manna and R Suresh "Chemometrics Assisted Rp-Hplc Method Development For The Separation Of Second Generation Tyrosine Kinase Inhibitors In Bulk Drugs And Pharmaceutical Formulations" *Int. Res. J. Pharm*. **2018**, 9 (1), 81-88.
[40]. Ramana Reddy , and Arthanareeswari M. "A stability indicating method development and validation for separation of process related impurities and characterization of unknown impurities of tyrosine kinase inhibitor Nilotinib by RP-HPLC, NMR spectroscopy and ESI-MS". *Int. Science Direct* **2020**, 8(4), 1-12.
[41]. G. Sowjanya, M. Mathrusri and A. Venkata "Development and Validation of a Stability Indicating RP-HPLC Method for the Determination of Nilotinib (A Tyrosine Kinase Inhibitor)" *Indo American Journal of Pharmaceutical Research,* **2013**, 3(6), 4541-4551.
[42]. "Selumetinib" April, 2020, https://go.drugbank.com/drugs/DB11689
[43]. For Drug "Selumetinib" May, 2020, https://www.drugs.com./mtm/selumetinib.html
[44]. Drug "Selumetinib" 2020, https://pubchem.ncbi/nlm/nih.gov/compound/selumetinib
[45]. United State Pharmacopeia, 2020, Vol II, pp-4030