Research Article

Stability Indicating RP-HPLC Method for the Estimation of Selumetinib in Capsule Dosage Form

Kamlesh Kashinath Saindane [1*], Harshada Talapadatur [2], Vijay Kumar Munipalli [2], Raman Mohan Singh [2], Bharti Fegade[1], Vaidun Bhaskar [1]

1. Department of Quality Assurance, Gahlot Institute of Pharmacy, Plot no. 59, Sector-14, Koparkhairne, Navi Mumbai, Maharashtra, India.
2. Department of Analytical Research and Development, Central Drug Testing Laboratory, Zonal FDA Bhavan, GMSD Compound, Belasis Road, Mumbai Central, Mumbai, Maharashtra, India.

***Corresponding author's E-mail:** kamleshsaindane322@gmail.com

Received: 12-03-2022; **Revised:** 24-05-2022; **Accepted:** 02-06-2022; **Published on:** 15-06-2022.

ABSTRACT

The principal objective of this study is to develop and validate a new, simple, accurate, fast, economical, precise, and reproducible Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) for the estimation of selumetinib in a capsule dosage form. The chromatographic separation was performed on the Inert Sustain C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column and a mobile phase comprising of 0.1 % Trifluoroacetic acid in Water and Acetonitrile in the ratio of (60:40 % v/v) was used. The diluent consisting of water and acetonitrile in the ratio of (50:50 % v/v) was used. The flow rate was kept at 1.1 ml/min and detection was carried out at 258 nm. The retention time of selumetinib was found to be about 5.9 min. The parameters such as accuracy, precision, linearity, ruggedness, robustness and LOD, LOQ were used for validating the developed method according to ICH guidelines. The method was linear over a concentration range of 50-175 µg/ml with a regression coefficient of 0.9998. Limit of Detection and Limit of Quantitation values for selumetinib was found to be 0.15 µg/ml and 0.47 µg/ml, respectively. The percentage RSD of every parameter was found within the limit. The stress testing studies were executed to give degradation products by exposing the drugs to hydrolytic, photolytic, oxidative, acid, alkali, and thermal degradation conditions. The acquired data showed that the degradation product successfully separated without any intrusion, which establishes the stability-indicating nature of a developed method. The accurate, simple, precise, economical, reliable, and easy for RP-HPLC method has been successfully developed and validated. The developed method was applied for routine quality control analysis of selumetinib in capsule dosage forms.

Keywords: Selumetinib, RP-HPLC, Method Development, Validation, Forced Degradation Studies.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v74i02.026

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v74i02.026

INTRODUCTION

Selumetinib is an Anti-Cancer drug used for the treatment of children, two years of age and older, with neurofibromatosis type 1(NF-1), a genetic disorder of the nervous system causing tumors to grow on nerves.[1] Selumetinib is chemically 6-(4-Bromo-2-chloroaniline)-7-fluoro-*N*-(2-hydroxyethyl)-3-methyl benzimidazole-5-carboxamide [fig.1], and its Molecular formula is $C_{17}H_{15}BrClFN_4O_3$.[2]

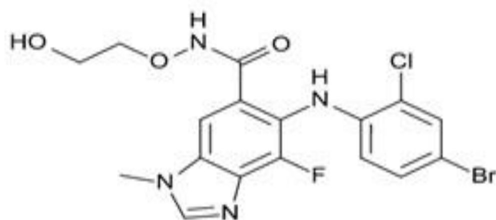

Figure 1: Chemical Structure of Selumetinib

Selumetinib is in a class of medications is also known as protein kinase inhibitors. It works by blocking the abnormal protein that signals the tumors to grow. This helps to stop or slow tumor growth.[3,4] It is approved specifically for children who have symptomatic, inoperable plexiform neurofibromas (PN), which are tumors involving the nerve sheaths (coating around nerve fibers) and can grow anywhere in the body, including the face, extremities, areas around the spine, and deep in the body where they may affect organs.[5-7]

Selumetinib is used to treat neurofibromatosis type 1 in children 2 years of age and older who have plexiform neurofibromas (PN; soft tumors).[8] Between 30 % and 50 % of children born with NF-1 develop one or more PNs.[1] That cannot be completely removed by surgery. This is a rare, progressive condition caused by a mutation or flaw in the gene coding for the protein neurofibromin 1.[9] NF-1 is diagnosed in early childhood and appears in an estimated one out of every 3,000 infants. It is characterized by changes in skin coloring (pigmentation), neurologic and skeletal impairments, and risk for the development of benign and malignant tumors throughout life.[1]

Literature survey discovered that the method available for selumetinib estimation is a Novel LC-MS method that has been developed for the determination of selumetinib.[10] There is no method for the estimation of selumetinib in

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

capsule dosage form by using the RP-HPLC method. There is wide scope for the development of a new analytical RP-HPLC method for the estimation of selumetinib in capsule dosage form.[11-13] Therefore, this study aims to develop and validate a new, simple, accurate, fast, economical, precise, reproducible RP-HPLC method for the estimation of selumetinib in capsule dosage form.[14]

MATERIALS AND METHODS

Chemicals and reagents

An analytically pure selumetinib working standard was procured from the Central Drug Testing Laboratory, Mumbai with defined potency [99.70 % as is basis]. Koselugo containing 10 mg and 25 mg selumetinib marketed by Astra Zeneca formulation was used for analysis. Acetonitrile (HPLC grade) from Merk life science, trifluoroacetic acid AR Grade from Merck, Ultra-purified HPLC grade distilled water was obtained from the Milli-Q®.[15-16]

Instrumentation

Perkin Elmer UV/VIS spectrometer lambda 25 connected to a computer loaded with software Perkin Elmer UV win lab was used for all the spectrophotometric measurements. The chromatography was performed on the instruments Thermo Scientific Ultimate 3000 system using chromeleon 7.4.2 software. Different types of apparatus like analytical weighing balance, vacuum filter pump, millipore filtration kit, sonicator, Water bath, sample filtration assembly, and glassware were used throughout the experiment.[17]

Chromatographic conditions

The chromatographic separation was performed on the Inert Sustain C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column and a mobile phase comprising of 0.1 % trifluoroacetic acid in water and acetonitrile in the ratio of (60:40 % v/v) was used. The diluent consisting of water and acetonitrile in the ratio of (50:50 % v/v) was used. The flow rate was kept at 1.1 ml/min. The injection volume of 10 µl and detector wavelength at 258 nm was selected.[18,19]

Determination of wavelength

Selumetinib standard 10 mg weighed accurately transferred to the 100 ml volumetric flask and the volume was made up to the mark with diluent (100 µg/ml). Then the solution was scanned in UV visible Spectrophotometer in the range of 400.0 nm to 220.0 nm. Selumetinib showed maximum absorbance at 258 nm as shown in Fig. 2. Hence the same wavelength was selected for the analysis of the selumetinib.[20]

Selection of solvent (diluent)

Based on the molecular structure and chemical nature of selumetinib, the water and acetonitrile in the ratio of (50:50 % v/v) were selected as a diluent for the preparation of standard and sample solutions.

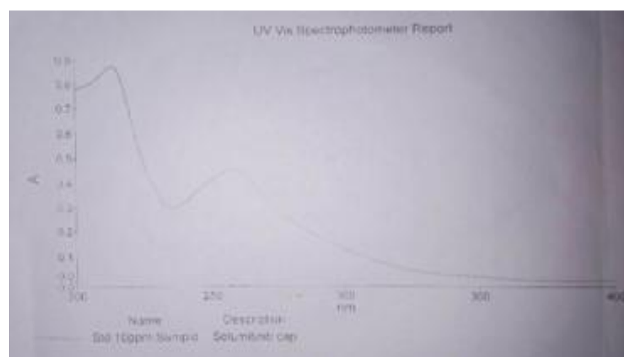

Figure 2: UV Spectra of Selumetinib

Preparation of Mobile Phase

Acetonitrile and 0.1 % trifluoroacetic acid in water in the ratio of 60:40 % v/v were used as a mobile phase for the present study. Trifluoroacetic acid (0.1 % v/v) was prepared by dissolving 1 ml of trifluoroacetic acid into 1000 ml of HPLC grade water. The mobile phase was vacuum filtered through 0.45 µm high flow nylon membrane filters purchased from Axiva Sichem Pvt. Ltd and was sonicated using an ultra sonicator.

Preparation of standard solution

A standard solution of 100 µg/ml was prepared using diluent.

Preparation of sample Solution

Four capsules of SELUMETINIB (10 mg) where the whole capsule i.e., 40 mg was dissolved in a diluent and sonicated for 10 min. Final dilution was made up to 100 ml with diluent (400 µg/ml) mixed thoroughly and then filtered. Then 5 ml from the above stock solution was diluted up to 20 ml with the same diluent (100 µg/ ml).

Method development

The chemical structure of the selumetinib shows that the drug is basic hence base deactivated column is the first choice to make trials for the reasonable retention of the drug. Different makes/brands of BDS columns with varying proportions of mobile phase comprising of water and organic solvents were studied. Initial trials were started with Water and Acetonitrile of different ratios were used but high tailing factors with long retention times were observed. Better peak shape with acceptable SST parameters was found on Inert Sustain C18 (250 mm × 4.6 mm i.d., 5 µm particle size) column with mobile phase comprising of 0.1% trifluoroacetic acid in water and acetonitrile in the ratio of (60:40 % v/v). The flow rate was kept at 1.1 ml/min and UV detection wavelength of 258 nm and column oven temperature maintained at 40 °C.[17-20]

Method validation

Validation of the developed method was done as per the ICH Q2 (R1) 22 guidelines concerning various parameters such as linearity, accuracy, LOD, LOQ, precision, and robustness. A standard solution of selumetinib was used for the comparison of results.[21-24]

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

System Suitability Testing

System suitability parameters were evaluated and analyzed to check the system performance by injecting a working standard solution (six replicate) of a concentration of 100 µg/ml into the HPLC. The chromatograms were recorded to evaluate SST parameters like % RSD of Area, retention time and tailing factor, theoretical plates.[25]

Linearity

The linearity of the method was determined by appropriate aliquots from standard selumetinib stock solutions were prepared to obtain concentrations of 50 - 175 µg/ml. The linear calibration plot was constructed by analyzing the concentrations over the selected range versus the peak area of the sample. The peak areas were noted by injecting each level into the chromatographic system. The response for the drug was linear in the concentration range between 50 - 175 µg/ml. The linearity was observed in the expected concentration range, demonstrating its suitability for analysis.

Accuracy

Accuracy is a measure to close is the experimental value to the true value. Good recovery of the spiked drug was obtained at each added concentration, to shows that the method was accurate. Accuracy was resolute by the method of standard addition method. A known amount of selumetinib (110 %, 120 %, and 130 %) of the standard solution was added to the pre-analyzed solution of the formulation. This solution was analyzed as previously described. The assay was repeated over 3 injections of each concentration was recorded the chromatograms and measured the peak responses were. Calculated the amount found and the amount added for selumetinib and calculated the individual recovery and mean recovery values. The resultant % RSD for this study was found to be < 2.0 % with a corresponding percentage recovery value.[20]

Precision

System Precision

This was performed by injecting six replicate injections of a standard solution (100 µg/ml). The average, SD, % RSD of six replicate injections was calculated and reported.

Method Precision (Assay Repeatability)

This was performed by injecting six replicate injections of standard solution (100 µg/ml) and six sample preparations of selumetinib (100 µg/ml) in triplicates into the HPLC system. Its % assay, average, SD, % RSD were calculated and reported.

Intermediate Precision

This was performed on two different days and different HPLC instruments. Five replicates of standard solution (100 µg/ml) and three sample preparation (100 µg/ml) in triplicates were injected into the HPLC system. Its % Assay, average, SD, % RSD were calculated and reported.

Robustness

It is defined as a small or deliberate change in the parameter that should not affect any method. This was performed by a change in flow rate (± 0.2 ml/min), change in the mobile phase composition (± 2 %), change in wavelength (± 2 nm), and % assay was calculated. The results had been within specified limits.[21]

Limit of detection

The selumetinib limit of detection (LOD) values was quantitated by using the formula -

$LOD = 3.3 \times \sigma \div s$

Where,

σ = Standard deviation of the intercept, s = slope of the calibration curve.[22]

Limit of quantitation

The quantitation limit (LOQ) of an analyte in the samples was quantified by using the formula-

$LOQ = 10 \times \sigma \div s$

Where,

σ = Standard deviation of the response, s = slope of the calibration curve.[22]

Force Degradation studies

Forced degradation is a degradation of substance and drug products at various stress conditions more severe than course conditions. It is required to reveal the specificity of stability indicating methods and degradation products of the drug substance and helps in explicating the structure of the degradation products.

The selection of stress conditions should be compatible with the product's decomposition under normal manufacturing, storage, and use conditions which are specified in each case. It is important to know when to perform forced degradation studies for the development of new drug substances and new drug products. FDA guidelines state that stress studies need to be performed in phase III of the regulatory submission process. Stress conditions need to be performed in different pH solutions, in the presence of oxygen and light, and at elevated temperatures and humidity levels to resolve the stability of the drug substance.[26]

Acid degradation

Added 10 ml of 2 N HCl to 10 ml of selumetinib stock solutions and heated it on boiling water bath at 60 °C for 2 hrs. Later the solution was cooled and neutralized to pH 7 with 2 N NaOH and diluted to volume with diluent to obtain 100 µg/ml solutions. Afterward filtered by a 0.45 µm membrane filter. The sample was injected into the HPLC system and represented the chromatograms.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Alkaline degradation

Added 10 ml of 2 N Sodium hydroxide to 10 ml of selumetinib stock solutions and heated it in a boiling water bath at 60 °C for 2 hrs. Later the solution was cooled and neutralized to pH 7 with 2 N HCL and diluted to volume with diluent to obtain 100 µg/ml solutions. Afterward filtered by a 0.45 µm membrane filter. The sample was injected into the HPLC system and represented the chromatograms.

Oxidation degradation

Added 10 ml of 3 % hydrogen peroxide to 10 ml of selumetinib stock solutions and heated it on boiling water bath at 60 °C for 2 hrs. Later the solution was cooled and diluted to volume with diluent to obtain 100 µg/ml solutions. Afterward filtered by a 0.45 µm membrane filter. The sample was injected into the HPLC system and represented the chromatograms.

Thermal degradation

Added 10 ml of selumetinib standard stock solutions and heated it on boiling water bath at 60 °C for 2 hrs. Later the solution was cooled and diluted to volume with diluent to obtain 100 µg/ml solutions. Afterward filtered by a 0.45 µm membrane filter. The sample was injected into the HPLC system and represented the chromatograms.

Water degradation

Added 10 ml of water to 10ml of selumetinib standard stock solutions and heated it on boiling water bath at 60 °C for 2 hrs. Later the solution was cooled and diluted to volume with diluent to obtain 100 µg/ml solutions. Afterward filtered by a 0.45 µm membrane filter. The sample was injected into the HPLC system and represented the chromatograms.

Photolysis degradation

Added 10ml of Selumetinib standard stock solutions were exposed to UV light at 254 nm for 24hrs. Later the solution was cooled and diluted to volume with diluent to obtain 100 µg/ml solutions. Afterward filtered by a 0.45 µm membrane filter. The sample was injected into the HPLC system and represented the chromatograms.

RESULTS AND DISCUSSION

System suitability

All the SST parameters like theoretical plates were observed greater than 7000 of selumetinib drugs. The peak tailing should be less than 2. The results are brief in Table No. 1.

Linearity

Linearity was determined over the range of (50-175 µg/ml) for the selumetinib. Regression equation obtained was y = 51893x + 29224. The method is having good linearity (r^2 = 0.9998). The results established that the analyte response is proportional to the analyte concentration in the selected concentration range. The calibration graphs of Selumetinib are depicted in fig. 5 and data in Tables 2, respectively.

Accuracy and recovery

Accuracy results at various levels of concentration are summarized in Table No. 3. For accuracy studies, the limit for percent means recovery is 110 %, 120 %, 130 %. From the results, it can be seen that the percent mean recovery is 100.58 % which is within the limit, hence the method is accurate.

Precision

The mean assay percentage results are brief in Table No. 4 and are found to be within the limit. The % assay, average, SD, % RSD for Day-1 and Day- 2, and HPLC-1 and HPLC- 2 were found to be not more than 2 %. The results are brief in Tables No. 5 and 6.

Robustness

By analyzing robustness, resulting values were found to be within the limit that is less than 2 %, thus the developed method was proved to be robust. The results are brief in Table No. 7.

LOD and LOQ

The present method can detect and quantify the analyte at a lower concentration. Values were estimated as following σ = 2413.28, S = 51892.94, LOD = 0.15 µg/ml, LOQ = 0.47 µg/ml. The results are brief in Table No. 8.

Assay

The % Assay of selumetinib in Koselugo tablets is 101.21 %, respectively, and the results are depicted in Table No. 9.

Degradation Studies

Forced degradation studies According to the ICH guidelines, stress testing was carried out upon exposure to extreme stress conditions of acid, base, peroxide, thermal, UV, and hydrolytic degradation. Later studied the main peak of the drug for peak purity by calculating the percentage of degraded amount and percentage of the active amount Forced degradation is carried out to produce representative samples for developing stability-indicating methods for drug substances and drug products.[26]

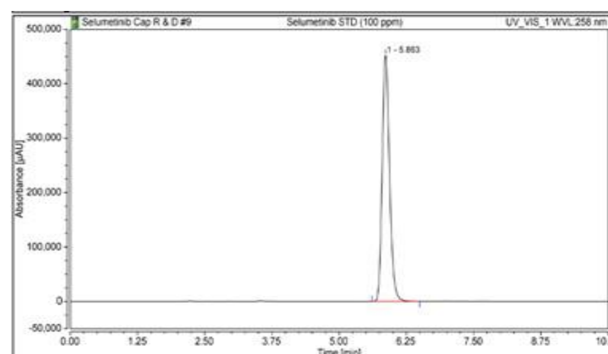

Figure 3: Chromatogram of Standard Sample Solution of Selumetinib

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

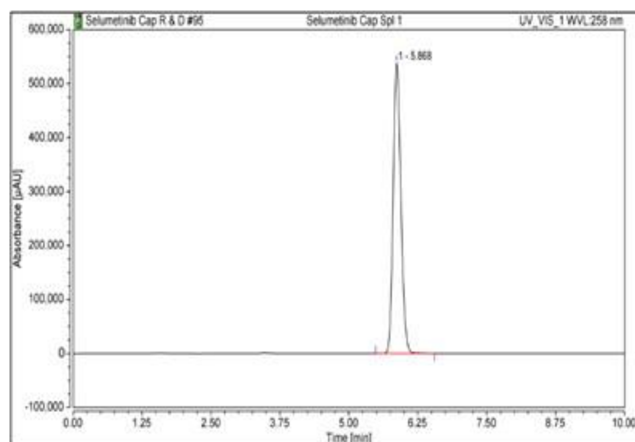

Figure 4: Chromatogram of Solution of Selumetinib

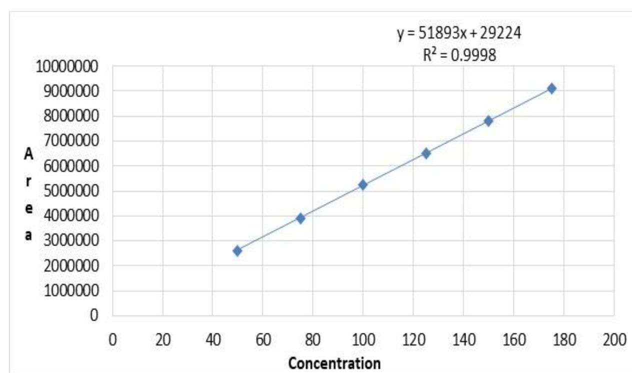

Figure 5: Linearity graph of Selumetinib

Table 1: System Suitability and System precision of Selumetinib

Sr no.	Area
1	5204383
2	5208236
3	5207252
4	5203929
5	5205856
6	5210265
Average	5206653.5
SD	2413.28
%RSD	0.05
Limit	NMT 2%

Table 2: Linearity data of Selumetinib

Linearity Level	Concentration µg/ml	Average Area Peak
1	50	2615733
2	75	3910990
3	100	5245720
4	125	6518349
5	150	7808058
6	175	9104231

Table 3: Accuracy data of Selumetinib

% LEVEL	Amount Spiked (ml)	Amount Recovered (mg/tab)	% Recovery	Mean % Recovery	SD	%RSD
110	1	11.26	102.74			
110	1	11.34	102.86	102.84	0.0957	0.0931
110	1	11.53	102.93			
120	2	11.87	99.77			
120	2	11.93	99.80	99.80	0.0238	0.0238
120	2	11.98	99.82			
130	3	12.82	99.06			
130	3	12.85	99.13	99.11	0.0466	0.0471
130	3	12.89	99.15			

Table 4: Method Precision

Sr no.	Area
1	5204383
2	5208236
3	5207252
4	5203929
5	5205856
6	5210265
Average	5206653.5
SD	2413.28
%RSD	0.05

Table 5: Intermediate Precision (INTERDAY) data of Selumetinib

Sr. No.	HPLC 1 - DAY 1 (%)	HPLC 2 - DAY 2 (%)
1	100.52	101.17
2	100.39	101.72
3	101.40	102.07
Average	100.77	101.65
S.D.	0.549	0.454
%R.S.D.	0.545	0.446
Limits	NMT 2.0%	NMT 1.0%

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

Table 6: Intermediate Precision (INTRADAY) data of Selumetinib

Sr. No.	10.00 AM (%)	1.00 PM (%)	4.00 PM (%)
1	98.17	98.71	98.11
2	98.85	97.59	97.77
3	98.62	97.87	97.07
Average	98.55	98.06	97.65
S.D.	0.346	0.583	0.530
%R.S.D.	0.351	0.594	0.543
Limits	NMT 2.0%	NMT 2.0%	NMT 2.0%

Table 7: Robustness data of Selumetinib

Parameter	Change in Parameter	% Estimation	Mean	SD	% RSD	Limit
Wavelength	256 nm	97.23	98.13	1.5704	1.6004	NMT 2.0%
	258 nm	99.94				
	260 nm	97.22				
Flow	0.9 ml/min	97.27	98.12	1.5743	1.6043	
	1.1 ml/min	99.94				
	1.3 ml/min	97.17				
Mobile Phase	59:39 %	97.04	97.98	1.6991	1.7341	
	60:40 %	99.94				
	61:41 %	96.96				

Table 8: Limit of Detection and Quantitation (L.O.D & L.O.Q) data of Selumetinib

Sr no.	Area
1	5204383
2	5208236
3	5207252
4	5203929
5	5205856
6	5210265
Average	5206654
S.D.	2413.28
%RSD (Limit NMT 2%)	0.05
Regression equation	y = 51893x + 29224
Slope	51892.94
L.O.D µg/ml	0.15
L.O.Q µg/ml	0.47

*Average Mean of Six determination, SD = Standard Deviation, % RSD = Percentage relative standard deviation.

Table 9: Assay Results of Selumetinib

Sample No.	Weight of standard (mg)	Sample weight (equivalent to 4 capsules i.e., 10 mg/capsule)	Mean Area of standard at 258 nm	Area of sample at 258 nm	% Assay
1	12.55	40	5180333	5185545	100.52
2		40		5178716	100.39
3		40		5231135	101.40
4		40		5219031	101.17
5		40		5247728	101.72
6		40		5265514	102.07
Mean					101.21
± SD					0.6611
% RSD					0.653

*Average Mean of Six determination, SD = Standard Deviation, % RSD = Percentage relative standard deviation, NMT = Not more than

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

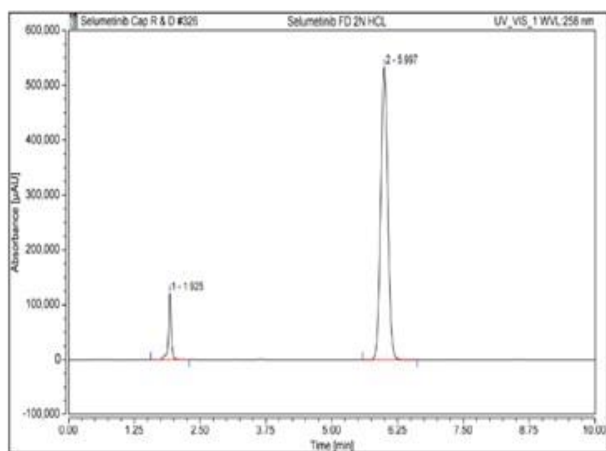

Figure 6: Acidic degradation

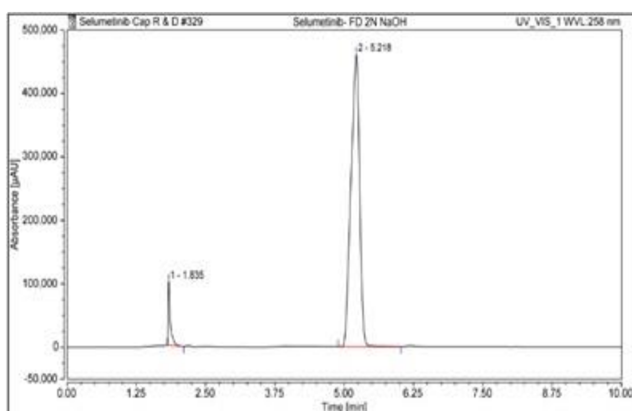

Figure 7: Alkaline degradation

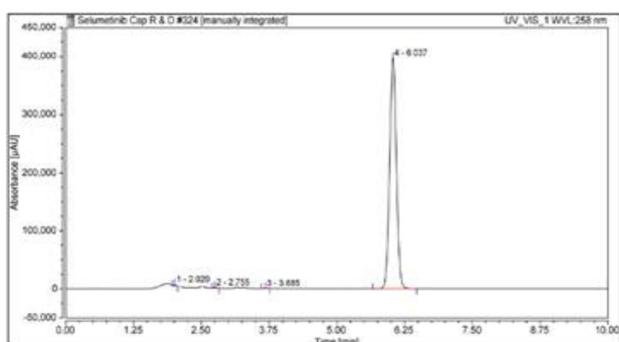

Figure 8: Oxidative degradation

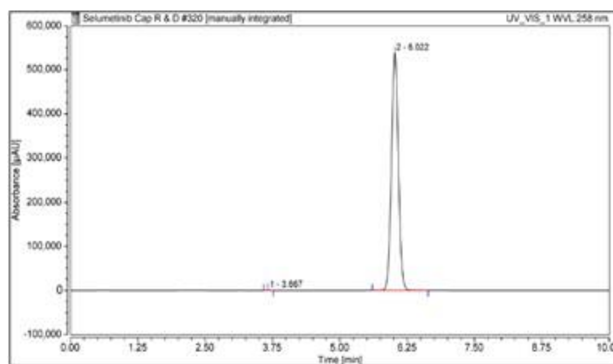

Figure 9: Thermal degradation

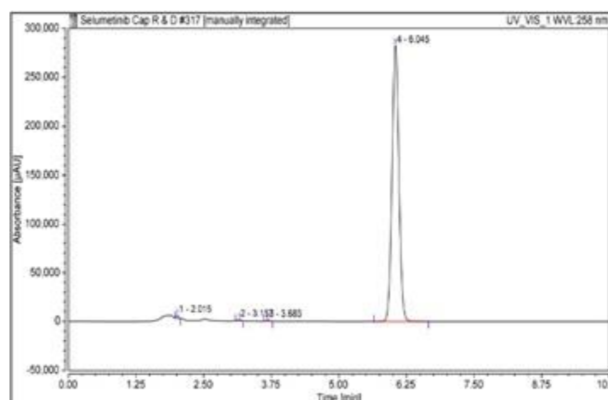

Figure. 10: Hydrolytic degradation

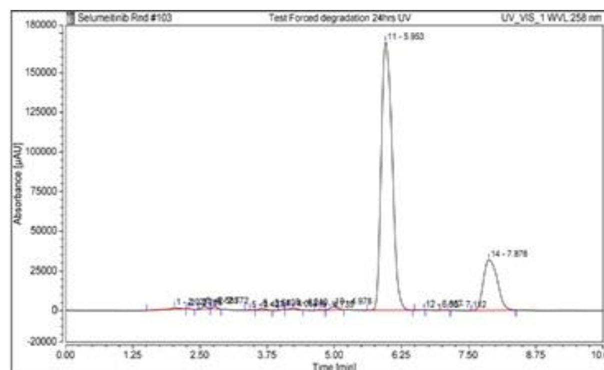

Figure 11: Photolytic degradation

Table 10: Force degradation studies data of selumetinib

Sr. no	Stress Condition	Duration (hrs)	Retention time of degradation products (Min)	% Residual Drug	Peak Purity (%)
1	Acidic 2 N HCL	2 hrs	1.925	91.81	99.99
2	Basic 2 N NaOH	2 hrs	1.835	95.07	100
3	Oxidative 3 % H_2O_2	2 hrs	2.020; 2.755; 3.685	99.47	100
4	Thermal	2 hrs	3.667	99.89	99.99
5	Water	2 hrs	2.015; 3.157; 3.683	99.12	100
6	Photolytic	24 hrs	2.027; 2.285; 2.585; 2.772; 3.427; 3.640; 4.058; 4.240; 4.735; 4978; 6.667; 7.112; 7.878	78.25	99.99

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

CONCLUSION

The RP-HPLC method development was found to be simple, selective, rapid, specific, and can generate accurate and precise results. Moreover, the shorter duration of analysis time and lesser mobile phase consumption confirmed that the method is rapid and economical. As per ICH Q2 (R1) guidelines, all the parameters that are linearity, accuracy and recovery, precision, specificity, robustness, and the assay are successfully validated. The successful separation of the forced degradation products from the active pharmaceutical ingredients without any interference confirmed the stability-indicating nature of the developed method. The parameters were within the range according to ICH guidelines. Hence the proposed RP-HPLC technique can be used for routine analysis and quality control of selumetinib in a capsule dosage form.

Acknowledgements: The author is thankful to the Director of Central Drug Testing Laboratory, Mumbai and Gahlot Institute of Pharmacy, koparkhairane for the guidance and support. Special thanks to Dr. Vijay Kumar, Mrs. Bharti Fegade, and Dr. V.H. Bhaskar. The authors are extremely grateful to Smt. S.U. Warde, Mrs. Harshada Talapadatur, Mrs. Ujwala, and others for their valuable guidance and support.

REFERENCES

1. FDA Approves First Therapy for Children with Debilitating and Disfiguring Rare Disease. U.S. Food and Drug Administration (FDA); 2020.
2. FDA approved Drug products, Koselugo [Selumetinib] Capsule for oral Use: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213756s000lbl.pdf
3. Gross Andrea M, "Selumetinib in children with Inoperable plexiform Neurofibromas", New England Journal of Medicines, 2020; 382(15): 1430-42.
4. Casaluce F, Sgambato A, Maione P, Sacco PC, Santabarbara G, Gridelli C. "Selumetinib for the treatment of non-small cell lung cancer". Expert opinion on investigational Drug. 2017; 26(8): 973-84.
5. Gross A, Wolters P, Dombi E. Selumetinib in Children with Inoperable Plexiform Neurofibromas. N Engl J. Med; 2020.
6. Dombi E, Baldwin A, Marcus LJ, Fisher MJ, Weiss B, Kim A, Whitecomb P, Martin S, Aschbacher- Smith LE, Rizvi TA, WU j, Ershler R, Wolters P, Therrein J, Glod J, Belasco JB, Schorry E, Brofferio A, Starosta AJ, Gillespie A, Doyle AL, Ratner N. Wideman BC: Activity of selumetinib in Neurofibromas. N Engl J Med. 2016; 375(26): 2550-2560.
7. "New Drug therapy approvals" U.S. Food and Drug Administration (FDA); 2020.
8. Urshina N, Rachana. Clinical application of circulating tumor DNA in early diagnosis of cancer. J of AJPCR. 2021; 14(3): 42.
9. Alimoradi H, Matikonda SS, Gamble AB, Giles GI, Greish K. Hypoxia responsive drug delivery system in tumor therapy. Curr pharm. 2016; 22(12): 2808-2820.
10. Voggu RR, Brus TS, Barksdale CT, Severin P, Hansen P, Chudnovskiy R, Thomas E, Bailey C. Novel LC-MS/MS method for the determination of selumetinib (AZD6244) in whole blood collected with volumetric absorptive microsampling. 2020; 12(13): 883-892. Doi: 10.4155/bio-2020-0062; PMID: 32628506.
11. S. Singh, P. Chaturvedi, S.K. Jain. Development and performance evaluation of tumor-targeting potential of folate spacer functionalized solid lipid nanoparticles. J of AJPCR. 2006; 14(6): 141-147. Doi: http://dx.org/10.22159/ajpcr.2021v14i6.40968
12. M. Chondhe, S. Hande, S. Singh. Evaluation and Validation of stability-indicating RP-HPLC method for the use of Berberine from coptis teeta bark as anti-cancer phytochemical. J of AJPCR. 2021; 14(4): 145-149.

    Doi: http://dx.org/10.22159/ajpcr.2021v14i4.40400
13. S. Madur, V. Matole, M. Kalshetti. UV-Visible Spectrophotometric method development and Validation of dasatinib in bulk and solid dosage form. J of IJCPR. 2020; 12(4): 90-93. http://dx.doi.org/10.22159/ijcpr.2020v12i4.39089
14. D.M. Rode, N.N. Rao. A review on development and validation of stability-indicating HPLC methods for analysis of Acidic drugs. 2019; 11(4): 22-33.

    Doi: http://dx.doi.org/10.22159/ijcpr.2019v11i4.34939
15. National Library of Medicine (U.S.), National Centre for Biotechnology Information, PubChem Compound Summary for CID 10127622, Selumetinib; 2004.
16. Koselugo (selumetinib). Wilmington, DE: AstraZeneca Pharmaceuticals LP; 2020.
17. Chatwal G, Anand S. Instrumental Method of Chemical Analysis. Himalaya publication. 2007; 10(5): 2.624-2.629.
18. P.D Sethi, Rajat Sethi, Santosh V. Gandhi, Nitin Dubey. High-Performance Liquid Chromatography, Sethi's Quantitative analysis of pharmaceutical formulation, volumes-5, CBS publisher & distributors pvt. Ltd.
19. Skoog, West, Holler, Crouch. Fundamental of Analytical chemistry, Eight edition; 973.
20. Mahadev B. Kshirsagar, P. Mahajan, Sanjay D. Sawant. Department of quality. Method development and validation by RP-HPLC for

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

estimation of drug in bulk and pharmaceutical dosage form.

21. Draft ICH Guidelines on validation of Analytical procedures definition and Terminologies. Federal Register, Vol 60. IFPMA, Switzerland. 1995; PP.1126.

22. ICH Stability Testing of New Drug Substances and Products Q1A[R2], Proceedings of International Conference on Harmonization; 2003.

23. International Conference on Harmonization, ICH Q1A [R], Stability Testing of new Drug substances and products; 2003.

24. M. Bakshi, S. Singh. Development of validated stability-indicating assay methods- a critical review. J. pharm. Biomed. Anal. 2002; 28(6): 1011-1040.

25. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC Method development, 2nd ed. Hoboken: John Wiley and Sons, Inc; 1997.

26. R. Singh, Z. Rehman. Current trends in forced degradation study for pharmaceutical product development. J. pharm Educ. 2002; 3(1): 54-63.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

For any question relates to this article, please reach us at: globalresearchonline@rediffmail.com

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.