Journal or Innovative Development in Pharmaceutical and Technical Science (JIDPTS)
Volume:9, Issue:11, Nov:2026

(J I D P T S)
ISSN(O):2581-6934

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ZIFTOMENIB IN BULK AND MARKETED PHARMACEUTICAL DOSAGE FORM

G. Srilakshmi[1]*, Kristadi Mithra Mutyamala[2], Bandari Naveen Kumar[3], Nishath Unissa[4]

[1]*Associate Professor, [2,3,4] Student

[1,2,3,4] Department of Pharmaceutical Chemistry,

[1,2,3,4] Teegala Ram Reddy College of Pharmacy, #4-202, Meerpet, Balapur (M), Ranga Reddy Dist, Telangana, India-500097

Corresponding Author : Dr. G. Srilakshmi, Associate Professor

Department of Pharmaceutical Chemistry

Teegala Ram Reddy College of Pharmacy, #4-202, Meerpet, Balapur (M), Ranga Reddy Dist, Telangana, India-500097

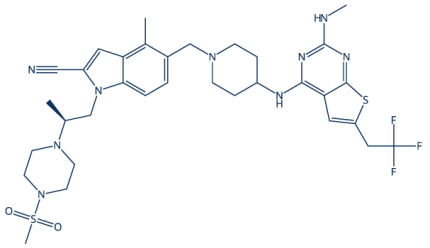
Abstract:

A new, simple, rapid, precise, accurate and reproducible RP-HPLC method for estimation of Ziftomenib in bulk form and marketed pharmaceutical dosage forms. Separation of Ziftomenib was successfully achieved on a Symmetry ODS RP C18, 5µm, 15cmx4.6mm i.d. column in an isocratic mode of separation utilizing Methanol : Phosphate buffer (0.01M, pH-3.4) in the ratio of 30:70% v/v at a flow rate of 1.0 mL/min and the detection was carried out at 255nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 12-28mcg/mL for Ziftomenib. The correlation coefficient was found to be 0.9995 for Ziftomenib. The LOD and LOQ for Ziftomenib were found to be 1.165µg/mL and 3.53µg/mL respectively. The proposed method was found to be good percentage recovery for Ziftomenib, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: Ziftomenib, RP-HPLC, Accuracy, Precision, Robustness, ICH Guidelines.

INTRODUCTION

Ziftomenib is a menin inhibitor used to treat acute myeloid leukemia with NPM1 mutations. Ziftomenib is a potent and highly selective menin inhibitor1. This drug was approved by the FDA on November 13th, 2025, for the treatment of relapsed or refractory acute myeloid leukemia (AML) in adults with a susceptible NPM1 mutation who have no satisfactory alternative treatment options2. Ziftomenib works by preventing the protein-protein interaction between menin and KMT2A, a complex essential for maintaining the proliferation and survival of these leukemic cells. Ziftomenib is indicated for the treatment of adult patients with relapsed or refractory acute myeloid leukemia (AML) with a susceptible nucleophosmin 1 (NPM1) mutation who have no satisfactory alternative treatment options3. The IUPAC Name of Ziftomenib is 4-methyl-5-[[4-[[2-(methyl amino)-6-(2, 2, 2-trifluoroethyl) thieno [2, 3-d] pyrimidin-4-yl] amino] piperidin-1-yl] methyl]-1-[(2S)-2-(4-methyl sulfonyl piperazin-1-yl) propyl] indole-2-carbonitrile. The Chemical Structure of Ziftomenib is as following



All rights reserved by www.jidps.com

Fig-1: Chemical Structure of Ziftomenib

After performing an extensive literature review, an attempt was made to develop a smooth plain sailing, unambiguous, valid, speedy, and decisive strategy for estimating Ziftomenib in bulk form and marketed pharmaceutical dosage form by using RP-HPLC31.

MATERIALS AND METHODS

Table-1: Instruments Used

S. No.	Instruments and Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA detector.
2	pH meter	Lab India
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Table-2: Chemicals Used

S. No.	Chemical	Brand names
1	Ziftomenib	Synpharma Research Lab, Hyderabad
2	Water and Methanol for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck
4	Ethanol	Sd fine-Chem ltd; Mumbai
5	DMSO	Sd fine-Chem ltd; Mumbai
6	DMF	Sd fine-Chem ltd; Mumbai
7	Orthophosphoric Acid	Sd fine-Chem ltd; Mumbai

HPLC Method Development:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.1ml of the above Ziftomenib stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Twenty capsules were taken and the average weight was calculated as per the method prescribed in I.P. The weighed tablets were finally powdered and triturated well. A quantity of powder of Ziftomenib equivalent to 10mg were transferred to clean and dry 10 ml volumetric flask and 7 ml of HPLC grade methanol was added and the resulting solution was sonicated for 15 minutes. Make up the volume up to 10 ml with same solvent. Then 1 ml of the above solution was diluted to 10 ml with HPLC grade methanol. One ml (0.1 ml) of the prepared stock solution diluted to 10 ml and was filtered through membrane filter (0.45µm) and finally sonicated to degas.

Procedure:

Inject the samples by changing the chromatographic conditions4 and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines13-14.

Mobile Phase Optimization:

Initially the mobile phase5 tried was Methanol and Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and Phosphate buffer (0.01M, pH-3.4) in proportion 30:70% v/v.

Optimization of Column:

The method was performed with various C18 columns like, X-bridge column, Xterra, and C18 column. Symmetry ODS RP C18, 5µm, 15cmx4.6mm i.d. column was found to be ideal as it gave good peak shape and resolution at 1.0ml/min flow.

Preparation of Buffer and Mobile Phase:

Preparation of Potassium Dihydrogen Phosphate (KH_2PO_4) Buffer (0.01M) (pH-3.4):

Dissolve 1.36086g of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.4 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration6 and ultra-sonication.

Preparation of Mobile Phase:

Accurately measured 300 ml (30%) of Methanol and 700 ml of Phosphate buffer (70%) were mixed and degassed in digital

ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

Method Validation Parameters

System Suitability

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ziftomenib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity:

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ziftomenib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution:

Weight 10 mg equivalent weight of Ziftomenib sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.1ml of Ziftomenib above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay7 by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity and Range:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (12ppm of Ziftomenib):

Take 0.12ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – II (16ppm of Ziftomenib):

Take 0.16ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – III (20ppm of Ziftomenib):

Take 0.2ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – IV (24ppm of Ziftomenib):

Take 0.24ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Preparation of Level – V (28ppm of Ziftomenib):

Take 0.28ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluents and sonicate the solution for bubble entrapment using ultrasonicator.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision

Repeatability

Preparation of Ziftomenib Product Solution for Precision:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and

make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ziftomenib stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:

For Preparation of 80% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.08ml of the above Ziftomenib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 100% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ziftomenib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

For Preparation of 120% Standard Stock Solution:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.12ml of the above Ziftomenib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:

Inject the Three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Ziftomenib and calculate the individual recovery8 and mean recovery values.

Robustness:

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Ziftomenib working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Ziftomenib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of Flow Conditions:

The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of Mobile Phase Organic Composition:

The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 35:65, 25:75 instead (30:70), remaining conditions are same. 20µl of

the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Selection of Wavelength:

The detection wavelength was selected by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200-400nm. The UV spectrum15 of Ziftomenib was obtained and the Ziftomenib showed absorbance's maxima at 255nm. The UV spectra of drug are follows:

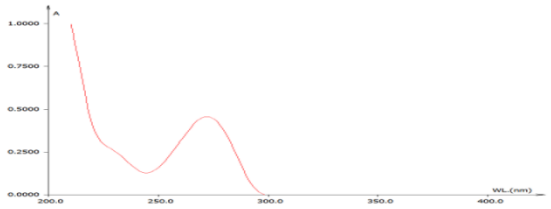


Fig-2: UV Spectrum of Ziftomenib

Observation: While scanning the Ziftomenib solution we observed the maxima at 255nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

Method Development

Several concurrent trails developed the proposed method to establish the preferred chromatographic conditions, which would be helpful to conduct a complete validation study. The optimal conditions considered for mobile phase were 30:70% v/v ratio of Methanol and 0.01M potassium dihydrogen orthophosphate buffer at pH 3.4 and Symmetry ODS RP C18, 5µm, 15cmx4.6mm i.d. Column16 were used, which gave a sharp symmetric peak, minimum tailing factor with short run time for Ziftomenib at a flow rate of 1.0 mL/min and detection wavelength 255 nm was optimized. Sample of 20µL was injected in to HPLC System17 and the different optimization of chromatographic parameters is depicted in Table 3. The retention time for Ziftomenib was found to be 3.213 minutes (Figure 3).

Optimized Chromatographic Method:

Table-3: Optimized Chromatographic Conditions

Mobile phase	Methanol: Phosphate buffer (0.01M, pH-3.4) = 30:70% v/v
Column	Symmetry ODS RP C18, 5µm, 15cmx4.6mm i.d.
Column Temperature	Ambient
Detection Wavelength	255 nm
Flow rate	1.0 ml/ min.
Run time	07 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	20µl
Type of Elution	Isocratic

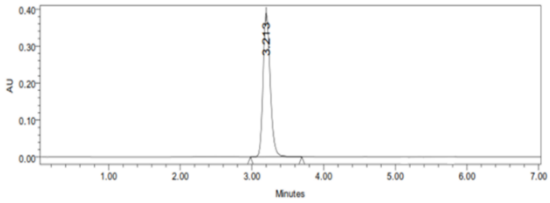


Fig-3: Optimized Chromatographic Condition

Conclusion: This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass the all-system suitability parameters. So, it's optimized chromatogram.

Validation of Analytical Method

System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters18 were established. The data are shown in Table-4 & 5.

Table-4: Data of System Suitability Test

S. No.	Injection No.	RT	Area	USP Plate Count	USP Tailing
1	Injection 1	3.253	284568	7368	1.26
2	Injection 2	3.254	285684	7295	1.25
3	Injection 3	3.215	283659	7346	1.27
4	Injection 4	3.297	284754	7394	1.29
5	Injection 5	3.253	283695	7425	1.25
6	Injection 6	3.213	284578	7385	1.27
Mean			284489.7	7368.833	1.265
S.D			752.5617		
%RSD			0.26453		

Table-5: System Suitability Results for Ziftomenib (Flow rate)

S. No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Ziftomenib = 0.12
2	Theoretical plate	N > 2000	Ziftomenib = 7258
3	Tailing Factor	(Tf) < 2	Ziftomenib = 1.25

Specificity:

Specificity19 can be determined by comparing the chromatograms obtained from the drugs with the chromatogram obtained from the blank solution. Blank solution was prepared by mixing the excipients in the mobile phase without drug. Drug solutions were prepared individually and the sample containing three drugs was also prepared. Now these mixtures were filtered by passing through 0.45 µ membrane filter before the analysis. In this observation no excipient peaks were obtained near the drug in the study run time. This indicates that the proposed method was specific. The chromatograms representing the peaks of blank, Ziftomenib and the sample containing the three drugs were shown in following figures respectively.

Observation: In this test method blank, standard solutions were analyzed individually to examine the interference. The above chromatograms show that the active ingredient was well separated from blank and their excipients and there was no interference of blank with the principal peak. Hence the method is specific.

Linearity: To evaluate the linearity20, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 0-28µg/ml for Ziftomenib. The prepared solutions were filtered through Whatman filter paper (No.41). From these solutions, 20µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve21 was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Plotting of Calibration Graphs: The resultant areas of linearity peaks are plotted against Concentration.

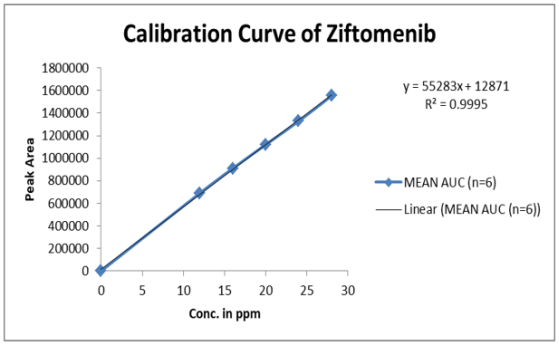


Fig-4: Standard Curve for Ziftomenib

Observation: Linearity range22 was found to be 0-28µg/ml for Ziftomenib. The correlation coefficient was found to be 0.9995, the slope was found to be 55283 and intercept was found to be 12871 for Ziftomenib.

Table-6: Linearity Readings for Ziftomenib

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
12	690316
16	910621
20	1121057
24	1328903
28	1554666

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Ziftomenib is a straight line.

Y = mx + c

Slope (m) = 55283

Intercept (c) = 12871

Correlation Coefficient (r) = 0.9995

Acceptan/Validation Criteria: ceThe response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 12871. These values meet the validation criteria.

Accuracy:

Inject the three replicate injections of individual concentrations (80%, 100%, 120%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Ziftomenib and calculate the individual recovery and mean recovery values23. Accuracy at different concentrations

(80%, 100%, and 120%) was prepared and the % recovery was calculated.

Table-7: Accuracy results of Ziftomenib

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Found	Conc. Recovered	Peak Area		
S1 : 80 %	8	8.064107	458679	99.867	Mean= 100.4113%
S2 : 80 %	8	7.843532	446485	100.637	S.D. = 0.473694346
S3 : 80 %	8	8.19449	465887	100.73	% R.S.D.= 0.471753
S4 : 100 %	10	9.892661	559767	99.41	Mean= 100.6646667% S.D. = 1.166369295 R.S.D.= 1.158667
S5 : 100 %	10	9.978655	564521	100.868	
S6 : 100 %	10	10.19623	576549	101.716	
S7 : 120 %	12	11.85907	668476	99.878	Mean= 100.4637% S.D. = 0.51154309 % R.S.D. = 0.509181
S8 : 120 %	12	12.16785	685546	100.69	
S9 : 120 %	12	12.18644	686574	100.823	

Observation: The mean recoveries were found to be 100.411, 100.664 and 100.463% for Ziftomenib. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Precision: The precision24 of each method was ascertained separately from the peak areas obtained by actual determination of six replicates of a fixed amount of drug Ziftomenib. The percent relative standard deviations were calculated for Ziftomenib are presented in the Table-8.

i) Repeatability

Obtained Six (6) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table-8: Repeatability Results of Ziftomenib

HPLC Injection Replicates	AUC for Ziftomenib
Replicate – 1	285479
Replicate – 2	284571
Replicate – 3	286954
Replicate – 4	283261
Replicate – 5	285964
Replicate – 6	284259
Average	285081.3
Standard Deviation	1318.666
% RSD	0.462558

Observation: The repeatability study which was conducted on the solution having the concentration of about 20µg/ml for Ziftomenib (n=6) showed a RSD of 0.462558% for Ziftomenib. It was concluded that the analytical technique showed good repeatability.

ii) Intermediate Precision / Ruggedness

To evaluate the intermediate precision25 (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

Analyst 1: The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Analyst 2:

The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits29-30.

Intra Day (Day-1)/Analyst-1:

Table-9: Results of Ruggedness for Ziftomenib (Analyst-1)

S. No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Ziftomenib	3.253	284568	7368	1.26
2	Ziftomenib	3.254	285684	7295	1.25
3	Ziftomenib	3.215	283659	7346	1.27
4	Ziftomenib	3.204	286598	7457	1.22
5	Ziftomenib	3.202	287965	7635	1.29
6	Ziftomenib	3.297	285698	7459	1.28
Mean			285695.		
Std.			1508.89		
% RSD			0.52814		

Inter Day (Day -2/Analyst-2)

Table-10: Results of Ruggedness for Ziftomenib (Analyst-2)

S. No.	Peak Name	RT	Peak Area	Theoretical Plates	Tailing Factor
1	Ziftomenib	3.297	294754	7394	1.29
2	Ziftomenib	3.253	293695	7425	1.25
3	Ziftomenib	3.213	294578	7385	1.27
4	Ziftomenib	3.297	296534	7584	1.23
5	Ziftomenib	3.210	296571	7745	1.24
6	Ziftomenib	3.254	298698	7658	1.25
Mean			295805		
Std. Dev.			1819.334		
% RSD			0.615045		

Robustness: Robustness26 is defined as the capacity of that method to be unaffected by even small deliberate changes that occur in the method parameters. The evaluation of robustness of a method is done by varying the chromatographic parameters such as pH, temperature, flow rate, mobile phase proportions change, ionic strength etc., and determining any possible effect on the results obtained by that method.

Table-11: Result of Method Robustness Test for Ziftomenib

Parameter used for Sample Analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	283261	3.254	7258	1.25
Less Flow rate of 0.9 mL/min	315864	3.297	7569	1.29
More Flow rate of 1.1 mL/min	298542	3.212	7841	1.41
Less organic phase	279856	3.253	7965	1.27
More organic phase	306985	3.215	7458	1.28

LOD: The limit of detection (LOD) is the lowest concentration of analyte in a sample which can be detected, but not quantitated. LOD27 is a limit test that specifies whether an analyte is above or below a certain value. Signal-to-noise ratio of three-to-one is used to determine LOD.

L.O.D. = 3.3 (SD/S).

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Table-12: Results of LOD

	LOD
SD of Intercept	19518.16286
Slope	55283

Observation: The LOD was found to be 1.165µg/ml for Ziftomenib.

LOQ: The Limit of Quantitation (LOQ) is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method. Signal-to-noise ratio of ten-to-one is used to determine LOQ28.

L.O.Q. = 10 (SD/S)

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

Table-13: Results of LOQ

	LOQ
SD of Intercept	19518.16286
Slope	55283

Observation: The LOQ was found to be 3.53µg/ml for Ziftomenib.

Assay of Pharmaceutical Dosage form

Twenty tablets/Capsules were taken and the I.P. method was followed to determine the average weight. Finally the weighed tablets are powdered and triturated well by using mortar and pestle. A quantity of powder which is equivalent to the 100mg of drugs were transferred to a clean and dry 100ml of volumetric flask and add 70 ml of mobile phase and the resulted solution was sonicated for 15 minutes by using ultra sonicator, Then the final volume was make up to the mark with the mobile phase. The final solution was filtered through a selected membrane filter (0.45 µm) and in order to sonicate to degas the mobile phase (Solvent system). From this above stock arrangement (1 ml) was exchanged to five distinctive 10 ml volumetric flagons and volume was made up to 10 ml with same dissolvable framework (Mobile stage).

The readied arrangements were infused in five repeats into the HPLC framework and the perceptions were recorded.

A duplicate injection (Blank Solution) of the standard arrangement likewise infused into the HPLC framework and the chromatograms and peak zones were recorded and figured.

ASSAY:

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

The assay was performed as explained in the previous chapter. The results which are obtained are following29-30:

Table-14: Recovery Data for estimation Ziftomenib in Komzifti Capsules

Brand name of Ziftomenib	Labelled amount of Drug (mg)	Amount (mg) found by the proposed method (n=3)	Assay %
Komzifti Capsules (Kura Oncology, Inc.)	200mg	199.569mg	99.748%

Observation: The amount of drug in Komzifti Capsules was found to be 199.569 (± 0.639) mg/tab for Ziftomenib & % Purity was 99.748 (± 0.398) %.

SUMMARY AND CONCLUSION

In the present investigation, a simple, sensitive, accurate, precise, and reproducible RP-HPLC method was successfully developed and validated for the quantitative estimation of the drug in bulk and marketed pharmaceutical dosage forms. The chromatographic separation was achieved using a Symmetry ODS RP C18 column (150 mm × 4.6 mm i.d., 5 µm particle size) with a mobile phase consisting of methanol and 0.01 M phosphate buffer (pH 3.4) in the ratio of 30:70% v/v. The flow rate was maintained at 1.0 mL/min, with UV detection at 255 nm, an injection volume of 20 µL, and a total run time of 7 minutes under isocratic elution conditions. The developed analytical method was validated according to ICH guidelines for various parameters, including system suitability, specificity, linearity, accuracy, precision, robustness, and solution stability. The linearity study demonstrated a satisfactory relationship between concentration and peak area, while accuracy studies confirmed acceptable percentage recovery. The precision studies, including repeatability and intermediate precision, showed percentage RSD values within the prescribed limits. Furthermore, the robustness study confirmed that the method remained reliable with minor deliberate variations in chromatographic conditions. The validation results demonstrated that all the evaluated parameters complied with the recommended acceptance criteria. Hence, the proposed RP-HPLC method was found to be reliable, economical, accurate, and reproducible and can be successfully employed for routine quality control analysis and quantitative estimation of the drug in bulk and marketed pharmaceutical dosage forms.

BIBLIOGRAPHY

[1]. https://go.drugbank.com/drugs/DB17171

[2]. https://pubchem.ncbi.nlm.nih.gov/compound/Ziftomenib

[3]. https://en.wikipedia.org/wiki/Ziftomenib

[4]. R. Snyder, J. Kirkland, L. Glajch, Practical HPLC Method Development, john Wiley and sons International publication, II Edn., 2011.

[5]. S. Ashutoshkar, Pharmaceutical Drug Analysis 2nd Edn, New Age International Private Limited Publishers, 452-474, 2005.

[6]. H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th End. C.B.S. Publishers and Distributors', New Delhi. 1-9, 157-167.

[7]. H.H. Williard, L.L. Merit, F.A. Dean, F.A. Settle, Instrumental Methods of Analysis, 6th Edn, C.B.S. Publishers and Distributors, New Delhi.: 430-440, 495-504,529-545.

[8]. B.K. Sharma, Instrumental Methods of Chemical Analysis. GOEL Publishing House, Meerut: 286-300.

[9]. Instant notes on analytical chemistry by D. Kealey and P.J. Haines, © BIOS Scientific Publishers Limited, UK, 6-7, 2002.

[10]. Gurdeep R. Chatwal, Sham K. Anand, Instrumental methods of Chemical Analysis,5th edition, Himalaya Publishing House(Mumbai), P-2.566, 2005,.

[11]. M. E. Swartz, Journal of liquid chromatography, 28(7/8), 1253-1263(2005).

[12]. Journal of Chromatography .B, Analytical Technologies in the Biomedical and life Sciences. 2008 March 1; 863(2): 258-265. Published on Jan 18 2008.

[13]. International Conference on Harmonization, Harmonized Tripartite Guideline. Validation of Analytical Procedures. Text and Methodology. Q2 (R1). November 2005.

[14]. International Conference on Harmonization (ICH). Validation of Analytical Methods: Definitions and Terminology. ICH Q2A. 1994.

[15]. J. M. Green, a practical guide to analytical method validation, anal. Chem. News & features, pp. 305a–309a, 1 May 1996.

[16]. P. A. Winslow and r. F. Meyer, defining a master plan for the validation of analytical methods, j. Validation technology, pp. 361–367, 1997.

[17]. Aoac peer-verified methods program, manual on policies and procedures, Arlington, Va., USA (1998).

[18]. R. Patil: J of Chromatographia, 67, 575, (2008).

[19]. Baht and Leena: J of Liq. Chrom., 30, 309, (2007).

[20]. H.H. Williard, L.L. Merit, F.A. Dean and F.A. Settle, Instrumental methods of analysis, 7th edition, C.B.S. Publishers, New Delhi, 2002.

[21]. GN Menon, LB White, Department of Analytical Research, Abbott Laboratories, (pub med-index for MEDLINE).

[22]. Food and Drug Administration (FDA), "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation;" Federal Register (Notices), Vol: 65 (169), 52776–52777, 2000.

[23]. Vibha G et al., Development and validation of HPLC method - a review. International Research Journal of Pharmaceutical and Applied Sciences. 2012, 2(4), 22-23.

[24]. Bliesner DM. In: Validating Chromatographic Methods. John Wiley & sons Inc. 2006, 88-92.

[25]. Development and validation of HPLC method - A Review, Vibha Gupta et al, International Research Journal of Pharmaceutical and Applied Sciences, 2012; 2(4):17-25.

[26]. A Review: HPLC Method Development and Validation, Santosh Kumar Bhardwaj *et al. International Journal of Analytical and Bioanalytical Chemistry, accepted 20 November 2015.

[27]. Method Development: A Guide to Basics Quantitative & Qualitative HPLC, LC, GC chromacademy.

[28]. Lalit V Sonawane* Bioanalytical Method Validation and Its Pharmaceutical Application- A Review Pharmaceutica Analytical Acta 2014, 5:3Center for Drug Evaluation and Research (CDER) Reviewer Guidance.

[29]. Validation of Analytical Procedures: Methodology. ICH-Guidelines Q2B, Geneva. 1996, 11. (CPMP/ICH/281/95).

[30]. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.

[31]. Ch. Sudheer*, Bioanalytical Method for the Estimation of Ziftomenib and Its Application to Pharmacokinetic Studies Using LC-MS/MS, Asian Journal of Pharmaceutical and Clinical Research, Vol 19, Issue 3, 2026, 97-102.