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

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

Dr. S. Naazneen[1], A. Pooja[2], Monika Stephenson[3], Ch. Shivani[4], A. Keerthana[5], Arshiya[6]

[1]Associate Professor & HOD, Department of Pharmaceutical Analysis, [2,3,4,5,6] Student,

[1]Corresponding Author: Dr. S. Naazneen

[1,2,3,4,5,6] St. Mary's College of Pharmacy, 9-1-248, St Francis Street, Secunderabad, Telangana-500025

ABSTRACT: Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the Quantitative Determination of Ziftomenib in active pharmaceutical ingredient and Marketed Pharmaceutical Dosage form. Methods: A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Ziftomenib. The chromatographic strategy utilized Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size, using isocratic elution with a mobile phase consists of Methanol and Phosphate Buffer (0.02M) (pH-3.8) was taken in the ratio of 70: 30% v/v. A flow rate of 1.0 ml/min and a detector wavelength of 245nm utilizing the UV detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines. Results: LOD and LOQ for the active ingredients were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness was determined as a part of method validation and the results were found to be within the acceptable range. Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drugs.

Key Words: Ziftomenib, RP-HPLC, Method Development, Validation, Accuracy, Precision.

INTRODUCTION

Ziftomenib is a menin inhibitor used to treat acute myeloid leukemia with NPM1 mutations. Ziftomenib is a potent and highly selective menin inhibitor. 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 options [1]. 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 options. Ziftomenib (brand name Komzifti) is an FDA-approved oral, once-daily menin inhibitor [2]. It is used specifically to treat adults with relapsed or refractory acute myeloid leukemia (AML) who have a susceptible nucleophosmin 1 (NPM1) mutation. This targeted therapy helps reduce immature leukemia cells and promotes the maturation of blood cells. 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 [3]. The Chemical Structure of Ziftomenib is shown in following figure-1.

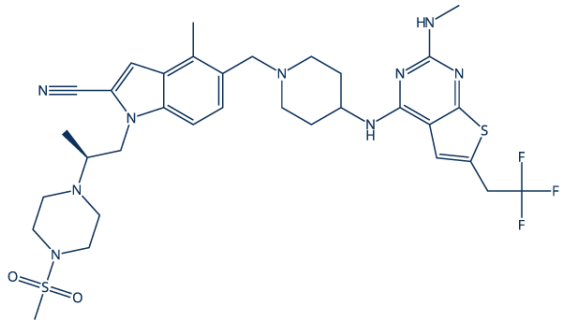

Fig-1: Chemical Structure of Ziftomenib

MATERIALS AND METHODS

Materials and Instruments:

The following are the list of instruments/Equipments, chemicals/reagents and standards to perform the HPLC Analysis of the drug Ziftomenib.

Equipment's:

Table - 1: List of Equipment's

S. No.	Instruments/Equipment's/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	T60-LABINDIA UV – Vi's spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry C18 Column, 250 mm x 4.6 mm and 5µm particle size
7.	PH Analyser (ELICO)
8.	Vacuum Filtration Kit (Labindia)

Chemicals and Reagents:

Table-2: List of Chemicals Used

S. No.	Name	Grade	Manufacturer/Supplier
1.	HPLC grade water	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	HPLC	Loba Chem; Mumbai.
3.	Ethanol	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	HPLC	Loba Chem; Mumbai.
5.	DMSO	A.R.	Sd fine-Chem ltd; Mumbai
6.	DMF	A.R.	Sd fine-Chem ltd; Mumbai

HPLC Instrumentation & Conditions: The HPLC system employed was **HPLC WATERS** with Empower2 Software with Isocratic with UV-Visible Detector [4].

Standard Preparation for UV-Spectrophotometer Analysis:

The Standard Stock Solutions – 10 mg of Ziftomenib standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with Methanol. Further dilutions were done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with methanol to get 10ppm concentration. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Ziftomenib, so that the same wave number can be utilized in HPLC UV detector for estimating the Ziftomenib [5].

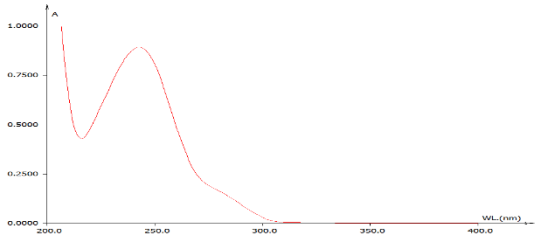

Fig-2: UV-Spectrum for Ziftomenib

Observation: While scanning the Ziftomenib solution we observed the maxima at 245nm.

Preparation of 0.02M Phosphate Buffer (pH-3.8): Prepare 800 mL of distilled water in a suitable container. Add 2.72172g of Potassium dihydrogen Phosphate to the solution to the solution. Adjust solution to final desired pH 3.8 using diluted solution of orthophosphoric acid and add distilled water until volume is 1 Litre.

Preparation of Mobile Phase: Mix a mixture of 0.02M Phosphate Buffer (pH-3.8) 700 ml (70%) and 300 ml Methanol HPLC (30%) and degas in ultrasonic water bath for 15 minutes. Filter through 4.5 µ filter under vacuum filtration [6].

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 out 0.1ml of Ziftomenib from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Different Trials for Chromatographic Conditions:

Table-3: Different Chromatographic Conditions

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Zorbax C18, 250 mm x 4.6 mm and 5µm Column	Methanol: Acetonitrile = 30: 70	0.9 ml/min	245nm	Extra peaks	Method rejected
Phenomenex C18, 250 mm x 4.6 mm and 5µm Column	Methanol: Acetonitrile = 60: 40	1.0 ml/min	245nm	Stabilization was not Good	Method rejected
Symmetry C18, 250 mm x 4.6 mm and 5µm Column	Methanol: Acetonitrile = 50: 50	1.0 ml/min	245nm	Improper peak separation	Method rejected
Symmetry C18, 250 mm x 4.6 mm and 5µm Column	Methanol: Phosphate Buffer (0.01M) (pH-2.8) = 40: 60	1.0 ml/min	245nm	Tailing peaks	Method rejected
Symmetry C18, 250 mm x 4.6 mm and 5µm Column	Methanol: Phosphate Buffer (0.02M) (pH-3.2) = 60: 40	1.0 ml/min	245nm	Tailing peaks	Method rejected
Symmetry C18, 250 mm x 4.6 mm and 5µm Column	Methanol: Phosphate Buffer (0.02M) (pH-3.8) = 70: 30	1.0 ml/min	245nm	Proper Peak	Method Accepted

Optimized Chromatographic Conditions:

Column : Symmetry C18, 250 mm x 4.6 mm i.d.5µm particle size

Mobile Phase : Methanol: Phosphate Buffer (0.02M) (pH-3.8) (70: 30% v/v)

Flow Rate : 1.0ml/minute

Wave length : 245 nm

Injection volume : 10 µl

Run time : 7 minutes

Column temperature : Ambient

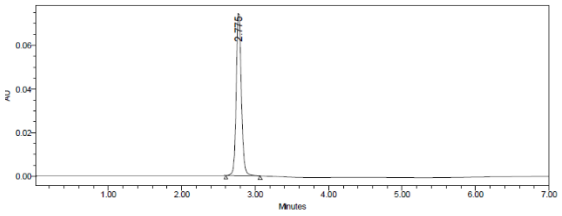

Fig-3: Optimized Chromatogram for Ziftomenib

Table-4: Results of Optimized Chromatogram

S. No.	Peak Name	Rt	Peak Area	Height	USP Tailing	USP Plate Count
1	Ziftomenib	2.775	715268	47844	1.35	5857

Method Validation:

System Suitability Test

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 parameters were established [7-9]. The data are shown in Table-5 & 6.

Table-5: Data of System Suitability Test

S. No.	Injection No.	RT	Area	Height	USP Plate Count	USP Tailing
1	Injection 1	2.786	715268	47844	5857	1.36
2	Injection 2	2.784	716584	46985	5986	1.38
3	Injection 3	2.768	715364	47258	5784	1.35
4	Injection 4	2.789	714895	47152	5896	1.34
5	Injection 5	2.784	716587	47258	5749	1.36
6	Injection 6	2.781	718549	47985	5657	1.39
Mean			716207.8		5821.5	1.36
S.D			1347.976			
%RSD			0.18821			

Table-6: Acceptance Criteria and Results

S.No.	Parameter	Limit	Result
1	Tailing factor	$T \leq 2$	1.36
2	Theoretical plate	$N > 2000$	5821.5

Accuracy:

Recovery Study: To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Ziftomenib were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation y = 74143x + 7294.9 [10]. The results were shown in table-7.

Table-7: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Mean % Recovery	
	Amount Injected	Amount Recovered				
S1 : 80 %	8	8.013	601425	100.162	Mean = 100.195%	
S2 : 80 %	8	8.012	601396	100.150		
S3 : 80 %	8	8.022	602123	100.275		
S4 : 100 %	10	10.038	751584	100.380	Mean = 100.356	% Mean Recovery = 100.364%
S5 : 100 %	10	10.039	751642	100.390		
S6 : 100 %	10	10.030	750969	100.300		
S7 : 120 %	12	12.057	901253	100.475	Mean = 100.541	
S8 : 120 %	12	12.073	902431	100.608		
S9 : 120 %	12	12.065	901864	100.541		

Observation: From the Accuracy Method, we observed that the mean %Recovery of the drug is 99.686 which are within the range of 98-102%.

Precision:

Repeatability:

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug Ziftomenib (API). The percent relative standard deviation was calculated for Ziftomenib [11-13].

Table-8: Results of Repeatability readings

HPLC Injection Replicates of Ziftomenib	Retention Time	Peak Area	Theoretical Plates	Tailing Factor
Replicate – 1	2.777	716984	5986	1.36
Replicate – 2	2.795	715698	5897	1.37
Replicate – 3	2.789	716859	5869	1.39
Replicate – 4	2.797	718548	5967	1.37
Replicate – 5	2.797	714895	5984	1.35
Replicate – 6	2.799	715986	5879	1.38
Average		716495	5930.333	1.37
Standard Deviation		1268.126		
% RSD		0.17699		

Observation: From the Precision method, we observed that the %RSD of the Peak Area is 0.176 which are within the acceptable range as per ICH guidelines [14].

Intermediate Precision:

The Intermediate Precision consists of two methods: -

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days [15].

Table-9: Peak results for Intra-Day Precision

S. No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Ziftomenib	2.784	716587	48685	1.38	5954	1
2	Ziftomenib	2.768	717845	48698	1.39	5935	2
3	Ziftomenib	2.786	716857	46989	1.36	5798	3
4	Average		717096.3	48124	1.376	5895.66	
5	S.D		662.2698				
6	% RSD		0.092354				

Table-10: Peak results for Inter-Day Precision

S. No.	Name	RT	Area	Height	USP Tailing	USP Plate	Injection
1	Ziftomenib	2.780	716987	49867	1.34	5968	1
2	Ziftomenib	2.794	718695	48574	1.33	5998	2
3	Ziftomenib	2.775	718542	48569	1.39	5859	3
4	Average		718074.7	49003.33	1.353333	5941.667	
5	S.D		945.0483				
6	% RSD		0.131609				

Observations: The intra & inter day variation of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Ziftomenib revealed that the proposed method is precise.

Linearity & Range:

To evaluate the linearity, serial dilution of analyte was prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 6-14µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis) [16-18].

Table-11: Linearity Concentrations of Ziftomenib

S.No.	Concentration (in ppm)	Peak Area
1	0	0
2	6	457896
3	8	607574
4	10	752268
5	12	896587
6	14	1036579

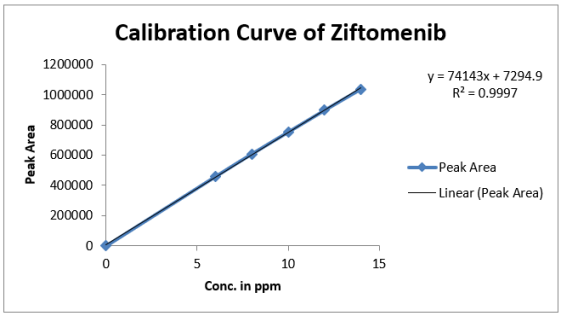

Fig-4: Calibration Curve of Ziftomenib

Observation: We observed that the calibration curve showed good linearity in the range of 6-14 µg/ml, for Ziftomenib with correlation coefficient (R2) of 0.9997. A typical calibration curve has the regression equation of y = 74143x + 7294.9 for Ziftomenib.

Specificity: Specificity of the pharmaceutical analysis is the ability to measure accurately and specifically the concentration of API, without interference from other active ingredients, diluents, mobile phase. Solutions of mobile phase, sample solution, standard solution were injected into liquid

chromatography. Retention times of samples and standard were compared [19-21].

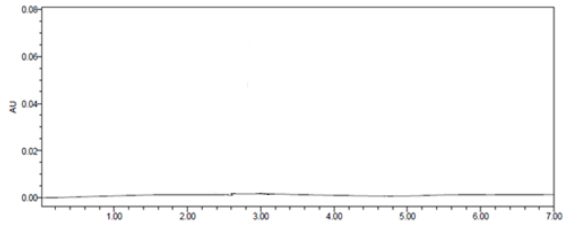

Fig-5: Chromatogram for Blank Solution

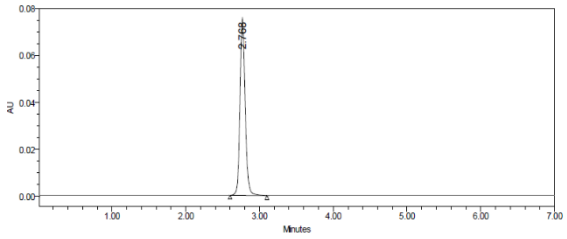

Fig-6: Optimized Chromatogram for Ziftomenib Standard

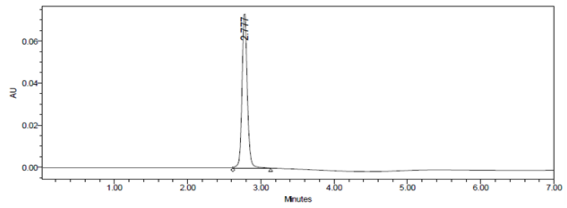

Fig-7: Optimized Chromatogram for Ziftomenib Sample

Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate 1ml (± 0.1ml/min), Wavelength of detection 245nm (±2nm) & organic phase content in mobile phase 60 (±5%) studied to determine the robustness of the method are also in favour of (% RSD <2%) the developed RP-HPLC method for the analysis of Ziftomenib (API) [22-25].

Table-12: Results of Method Robustness Test

Change in Parameter	Theoretical Plates	Tailing Factors
Flow (1.1 ml/min)	5954	1.35
Flow (0.8 ml/min)	6188	1.39
More Organic (70+5)	5748	1.41
Less Organic (70-5)	6185	1.48
Wavelength of Detection (250 nm)	6184	1.69
Wavelength of detection (240nm)	6247	1.47
Temperature (30 ^{0}C)	6324	1.34
Temperature (20 ^{0}C)	6985	1.32

LOD & LOQ: The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

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

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

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.507 & 1.539 µg/ml respectively [26-28]

Estimation of Ziftomenib in Pharmaceutical Dosage Form

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 10 mg of drug were transferred to 10 ml volumetric flask, and 8 ml of mobile phase was added and solution was sonicated for 15 minutes, there after volume was made up to 10 ml with same solvent. Then 1ml of the above solution was diluted to 10 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1.0 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded [29]. The data are shown in Table-13.

ASSAY

% Assay=AT/AS×WS/DS×DT/WT×P/100×AW/LC×100

Where:

AT = Peak Area of Ziftomenib obtained with test preparation

AS = Peak Area of Ziftomenib 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

Results obtained are tabulated below [30]:

Table-13: Assay of Ziftomenib in Pharmaceutical Dosage Forms

Brand name of Tablets/Capsules	Labelled Amount of Drug (mg)	Mean (±SD) Amount (mg) Found by the Proposed Method (n=5)	Assay + % RSD
Komzifti Capsules (Kura Oncology, Inc.)	200mg	199.564 (± 0.384)	99.457 % (± 0.387)

Observation: The %Purity of Komzifti Capsules containing Ziftomenib was found to be 99.475% (± 0.387).

SUMMARY AND CONCLUSION

A simple, rapid, and reliable RP-HPLC method was developed for the quantitative determination of Ziftomenib. The method was developed using a WATERS HPLC system with UV-Visible detection and a Symmetry C18 column (250 × 4.6 mm, 5 µm). Different chromatographic conditions were evaluated, and the optimized conditions provided a satisfactory peak shape and separation. The optimized chromatographic conditions consisted of Methanol and 0.02 M phosphate buffer adjusted to pH 3.8 in the ratio of 70:30% v/v, with a flow rate of 1.0 mL/min and detection at 245 nm. The injection volume was 10 µL and the total run time was 7 minutes. Under these conditions, Ziftomenib showed a retention time of approximately 2.775 minutes, with a tailing factor of 1.35 and a theoretical plate count of 5857. The developed method was validated for system suitability, accuracy, precision, intermediate precision, linearity, specificity, robustness, LOD, LOQ, and assay. The system suitability results showed a tailing factor of 1.36 and theoretical plate count of 5821.5, satisfying the reported acceptance criteria. The accuracy study showed recovery values within the specified 98–102% range, while repeatability demonstrated a %RSD of 0.176%, indicating good precision. The method exhibited good intermediate precision, with intra-day and inter-day %RSD values of 0.092% and 0.132%, respectively. Linearity was established over the concentration range of 6–14 µg/mL, with a correlation coefficient (R^2) of 0.9997 and regression equation of y = 74143x + 7294.9. The method was also reported to be specific and robust against small deliberate changes in chromatographic conditions. The LOD and LOQ of Ziftomenib were found to be 0.507µg/mL and 1.539 µg/mL, respectively, demonstrating the sensitivity of the proposed method. The developed method was successfully applied to the estimation of Ziftomenib in Komzifti capsules, where the reported assay was approximately 99.46%, indicating satisfactory drug content in the pharmaceutical dosage form.

In conclusion, the developed RP-HPLC method is simple, rapid, precise, accurate, sensitive, specific, and robust for the estimation of Ziftomenib in bulk drug and pharmaceutical dosage forms. The short 7-minute run time and satisfactory validation results indicate that the method can be effectively used for routine quantitative analysis and quality-control testing of Ziftomenib-containing pharmaceutical formulations.

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

[26]. 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.

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

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

[29]. 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.

[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.