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Development and validation of Stability Indicating RP-HPLC Method for the Determination of Axitinib in Bulk and its Pharmaceutical Formulations

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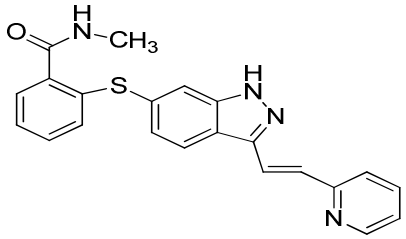
ABSTRACT

This paper presents a novel, simple, accurate and cost-effective Stability-indicating Reverse-Phase High-Performance Liquid Chromatography method for determination of Axitinib in bulk drug and forced degradation products in the pharmaceutical formulation was developed and validated. The current chromatographic separation was achieved with the Altima C18, (150 × 4.6 mm 5µm) column with a mobile phase used a mixture of acetonitrile and KH_2PO_4 (60:40, v/v %). The temperature of the column at 30°C with a flow rate of 1.0 ml/min and wavelength set at 338 nm. The retention time of axitinib was 4.27 min respectively. Drug product was subjected to stress conditions of acidic, alkaline, oxidative, thermal, hydrolytic, photochemical degradation. All the degradation products were well separated from axitinib it's highly sensitive towards acid, base and peroxide degradation. The developed method was validated statistically, parameters such as specificity, accuracy, precision, limit of detection, limit of quantification and robustness as per the ICH guidelines. The linearity dynamic range 25-250 µg/ml and effective mean percentage recoveries were 99.63% and LOQ, LOD values of axitinib were found to be 0.62 and 1.88 µg/ml, where recovery were 99.37% - 99.87 % respectively. Statistical analysis of the suggested method and reference method using student 't-test' and the F-ratio test, reveals that this suggested method demonstrated greater accuracy and sensitivity compared to the existing reference method. This method can be applied in different aspects like drug testing and routine analysis in quality control of pharmaceutical industries.

Keywords: Axitinib, HPLC, Stability-indicating method, Validation, Tablet.

INTRODUCTION

Axitinib abbreviated AXT, a small molecule indazole derivative, class of kinase inhibitor, a potent, selective and orally active therapeutic agent for the treatment of metastatic renal cell carcinoma (mRCC) and advanced renal cell carcinoma (RCC, a type of cancer that begins in the cells of the kidneys)[1-2]. AXT is an inhibitor of vascular endothelial growth factor receptor (VEGFRs)1-3. AXT prevents the kinase activity of VEGFR by binding to ATP-binding site of tyrosine kinase region and regulate HIF-1a-mediated tumor cell multiplication. Inhibits vascular permeability, angiogenesis and effective against breast tumors[3-4]. Chemically known *as N-Methyl-2 [[3-[(e) -2-pyridin-2-ylethyl-1h-indazole-6-yl] sulfanyl] benzamide*. The molecular formula is $C_{22}H_{18}N_4OS$ and mass 386.46; the structure is illustrated in [Figure 1]. Axitinib (Inlyta®) is approved for the United States, and other countries throughout the world for the second line treatment of patients with advanced renal cell cancer[5].



Till now, few articles have been published for the determination of AXT in pharmaceutical formulations and biological fluids, using different techniques. Quantification of axitinib in human plasma by LC-MS [6] Ion mobility spectrometry and DARTTM mass spectrometry by Identification of axitinib and HPLC [7-8]. Liposomal nano particles for systemic drug delivery and metabolism [9-10]. The extensive literature revealed, there is no official method in any of the pharmacopeias for determination of axitinib by HPLC. Stability studies at normal and stressed conditions play a major role in the determination of drug self-life and identification of degradation behavior. Stress studies and validation should be carried out on a drug as per ICH and USP guidelines [11-16].

The scientific novelty of present work is to establish a validated stability-indicating Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for estimation of Axitinib in pharmaceutical formulation and presence of its degradation products. Assessment of stability of its dosage forms (formulation) and purity of the bulk drug (API). Stability studies of Axitinib show the piece of information which will help in the formulation; it will open a new scope for the toxicity studies of degraded components. The proposed method is simple, accurate, rapid, reproducible and economical. The method is less time consuming and less expensive compared with the other published methods. The effective separation of all the degradants from the main peak and runtime was 8.0 minutes. So the present work used for determination of Axitinib in bulk and tablet dosage form, it can successfully use in stability studies and quality control laboratory for routine analysis.

MATERIALS AND METHODS

Chemicals and Reagents

Axitinib was obtained from Spectrum pharma solutions, Hyderabad, Telangana, India. It is a white amorphous powder, slightly soluble in water. Methanol (MeOH), Acetonitrile (ACN) HPLC grade, were purchased from Merck, (Mumbai, India). Analytical reagent (AR) grade Potassium dihydrogen phosphate (KH_2PO_4), Hydrogen peroxide (H_2O_2), Sodium hydroxide (NaOH), Orthophosphoric acid (H_3PO_3) Hydrochloric acid (HCl), were procured from SD Fine Chemicals (Mumbai, India). HPLC grade water was used for all preparation of samples and solutions.

Instrumentation

The analysis of samples was accomplished by HPLC (Waters e2695 Alliance, USA) instrument equipped with auto-sampler, quaternary gradient pump and an on-line degasser, column compartment with temperature control, Diode Array Detector (DAD) a dual wavelength detector. Chromatography data were obtained by using Empower-2 Software and separation carried with Altima C_{18} Column. HPLC grade water prepared by using Millipore Milli-Q water purified system Bedford, MA, USA.

HPLC Chromatograph conditions

The mobile phase composed of 60 volumes of Acetonitrile, 40 volumes of 0.01M KH_2PO_4, Separation was isocratic mode using the Column Altima C18, 150 x 4.6 mm i.d, 5 µm, injection volume was 10 µl with a flow rate of 1.0 ml/min, and detection was carried out at 338 nm using DAD and Column temperature maintained at 30ºC.

Preparation of Stock and working solution

AXT (1mg/ml) was prepared by adding 100 mg of the compound and 70 ml of the diluent (Diluent: The ratio 95 volumes of Acetonitrile and 5 volumes of water) in a volumetric flask, which was sonicated for 5 minutes and solution was made up to mark 100 ml with diluent. The working standard solution was 100 µg/ml by transferring 1.0 ml of the standard stock solution into 10 ml volumetric flask made up to the mark with diluent. Aliquots of the standard stock solution of AXT were transferred using a bulb pipette to 10 ml

volumetric flask, different concentrations of solutions were prepared as 25, 50, 75, 100, 125, 150, 200 and 250 µg/ml.

FORCED DEGRADATION STUDIES

Forced degradation study was carried on 100 µg/ml solution of axitinib, conditions such as acidic, basic, neutral, oxidation, photolysis, hydrolysis and thermal according to ICH guidelines. The concentration of the agents was initial with the lower concentration and increased to higher concentration to estimate the degradation behavior up to 2-20% and Stress samples are prepared using a mixture of the diluent and aqueous solutions.

Acid Degradation Studies

Acid degradation analysis was executed by 1.0 ml of AXT stock solution mixed with 1.0 ml of 0.5N, 1.0N, 2.0N HCl in to each volumetric flak and refluxed for 6 h at 60°C. The resultant solution was cooled to room temperature and neutralized with 0.5N, 1.0N, 2.0N NaOH and diluted to achieve final concentration 100µg/ml of the solutions.

Alkali Degradation Studies

Alkali degradation study was investigated by 1.0 ml of AXT stock solution mixed with 1.0 ml of 0.5N, 1.0N and 2.0 N NaOH and refluxed for 6 h at 60°C. The resultant solution was cooled to room temperature and neutralized with 0.5N, 1.0N and 2.0N HCl and diluted to achieve final concentration 100 µg/ml of the solutions.

Oxidative Degradation

Hydrogen peroxide-induced degradation study was executed by 30% of H_2O_2 of solution from that 1.0 ml and 1.0 ml of stock solution of AXT. The solutions were kept for 6 h at room temperature and neutralized with sodium thiosulphate. The solution was diluted to obtain final concentration 100 µg/ml solutions.

Dry Heat Degradation

The standard drug solution was placed in the oven at 105°C for 6 h to study the dry heat degradation. The solution was diluted to obtain final concentration 100 µg/ml.

Photo Stability Degradation

The photochemical stability of drug was also studied by exposing the 1000 µg/ml solution to Sunlight at 4 days. The resultant solution was taken 1.0 ml diluted to obtain final concentration 100 µg/ml solution.

Neutral Degradation

Stress testing under neutral condition was studied by refluxing the drug in water for 6 h at 60ºC resultant solution was diluted to final concentration 100 µg/ml solutions.

METHOD DEVELOPMENT AND OPTIMIZATION

The method development was done by pure AXT and formulations, samples were obtained from different degradation conditions in order to select the condition that would achieve the good resolution between the main compound and degradant products.

Optimization of mobile phase

Several parameters were evaluated for method development and optimization. Selection of suitable solvent, mobile phase and column is important for separation of AXT from the formulation, degradant products, and additives in a single run. In this study, DAD-detector is used for analysis. Here different compositions of mobile phase solvents are used in trial and error method. The mobile phase consisting acetonitrile and water in 50:50 v/v%, the ratio with a flow rate of 1.0 ml/min. The main peak was not eluted up to 45 min in currently used mobile phase and different mobile phase ratio as (ACN: H_2O, 70:30, 80:20, 90:10 and 65:45 v/v%). The result shows that elution late because drug ionization less. Then, introducing low pH buffers such as 0.1% orthophosphoric acid (OPA) and acetonitrile in 50:50 v/v%. The peak elution at the void volume (less than 1.0 min) as well as peak broadening, the USP theoretical plate count was found to be less than 2000. Then decrease the ratio of buffer to 40:60 v/v% observed that separation of split peaks. Then, alter the ratio of acetonitrile 35:65 and 28:72 v/v%, peaks were tailing respectively. When 0.1% OPA buffer

replaced with phosphate buffers, such as 0.01M KH_2PO_4 and acetonitrile used 50:50, v/v%, observed the peak shape good and retention time 3.83 min, asymmetry was found at 0.92. Consequently, the organic ratio was increased to 40:60, v/v% KH_2PO_4: ACN all peak shapes were good, retention time at 4.28 min, asymmetry was 1.24. The wavelength was set at 338 nm based on the UV spectra, AXT having two wavelengths such as 260.2 and 338.7 nm at the maximum absorption [Figure 2] was selected. Different temperature (25 and 35ºC) and flow rates were tried (0.8, 1.0 and 1.5 ml/min) and no changes were observed. System suitability parameters were within the limits as per ICH guidelines, all peaks were eluted with a single runtime (Rt) 8.0 min.

Figure 2: UV Spectrum of Axitinib, retention time 4.283 min, C=100 µg/ ml [absorption maxima, nm (1) 260.2 (2) 338.7

METHOD VALIDATION

The developed method was validated according to ICH and USP guidelines[11-13]. Parameters such as the limit of detection, limit of quantification, specificity, accuracy, precision, and robustness. System suitability conditions were tested the initial stage of the validation, injecting the five replication of standard preparation was injected and measured all the retention time, tailing factor, relative standard deviation and number of theoretical plates.

System suitability testing parameters

System suitability testing was performed during the method development and validation. The selectivity (α) and resolution (R_S) factors were calculated between AXT and nearest eluted peak. All the degradation condition asymmetry was found to be less than 1.5 [Figure 3] shows retention time and theoretical plates were calculated all values are acceptable limits in Table1.

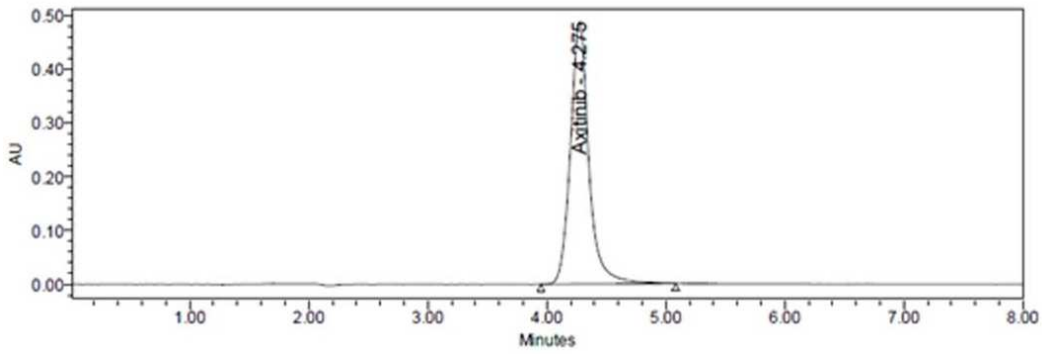


Figure 3: Chromatogram of Standard of Axitinib 100µg/ml.

Table 1: Results of system suitability parameter

Parameter	Acceptance criteria	Observed value	
		Precision (Day-1)	Intermediate precision (Day-2)
Retention time	-	4.283±0.14	4.281±0.18
Area(%RSD,N=6)	≤2.0	0.55	0.45
USP Plate count	>2000	4250	4165
USP Tailing	2.0	1.16	1.12

Mean ±%RSD for six determinations.

Linearity:

The linearity of this method is evaluated by linear regression analysis, which is calculated by the least square method and the drug is linear in the concentration range of 25-250 µg/ml. Calibration standards are prepared by spiking required volume of standard stock (1000 µg/ml) solution into different 10 ml volumetric flasks and volume made up with diluent to yield concentrations of 25, 50, 75, 100, 125, 150, 200 and 250 µg/ml and resultant peak area of drug was measured. Calibration curve is plotted between peak areas of the drug against the concentration of the drug. The linearity regression coefficient (r^2) including the slope and y-intercept, the correlation coefficient was 0.999 (Table 2).

Detection Limit and Quantification

In order to determine detection and quantification limits, drug concentration in the lower level of linearity range of the calibration curve. The equation LOD = 3.3×N/B and

LOQ = 10×N/B was used. Where n is the standard deviation of response and B slope of the corresponding calibration results were shown in (Table 2).

Table 2: Evaluation of LOD, LOQ and Linearity data

Parameter	Observed value
LOD (µg/ml)	0.62
LOQ (µg/ml)	1.88
Linearity range (µg/ml)	25-250
Correlation coefficient	0.9998
Slope	52638.8
Intercept	9954.4

Precision and Specificity

Interday and Intraday precision were carried in the replicating analysis. For interday precision, the analysis was done for three consecutive days at the same concentration level as used in intraday precision. The intraday precision was carried out at the various intervals time of the same day the percentage relative standard deviation (%RSD) and peak area were calculated. The specificity of the method was determined by separating degradation products from AXT (Figure 5). The degradation peaks were sharp, no baseline interferences and peak purity were higher than purity threshold (Table 7). This shows that there were no peak interferences in drug; precision data shown in (Table 3).

Table 3: Results from Evaluation of the precision and Intermediate precision of method

Parameter	Observed value	
	Precision (Day-1)	Intermediate precision (Day-2)
%Assay	100.89	100.46
%RSD (n=6)	0.5516	0.4525

Accuracy

Accuracy was calculated as the percentage recoveries of drug and determining different concentrations using standard addition method. The sample concentration was 100 µg/ml and covering the working standard range of drug 50%, 100%, 150% as the know concentration. The developed method was accurate, reliable and mean percentage recovery was 99.63%.The accuracy data was shown in (Table 4).

Table 4: Accuracy data of Axitinib

Target level (%)	Sample concentration (µg/ ml)	Amount of standard added (µg/ ml)	Total concentration (µg/ ml)	Found concentration (µg/ ml)	(%)RSD	Recovery mean
				98.72		
				99.64		
50	100	50	150	100.59	0.94	99.65
				100.75		
				98.00		
100	100	100	200	99.35	1.38	99.37
				100.37		
				99.07		
150	100	150	250	100.16	0.70	99.87

%RSD: Relative standard deviation

Robustness

The robustness conditions were optimized in order to identify critical parameters for the successful application. There is no significant difference observed when small deliberate changes were made for organic 2.0% variation calculated % RSD value, the flow rate at 0.1, wavelength ±10 and column temperature at 5°C. The retention time was observed 3.87±1.01. The robustness data was shown in (Table 5).

Table 5: The Robustness chromatographic parameters on RP-HPLC analysis of Axitinib

Change in parameter	%RSD
Flow (0.9 ml/min)	0.71
Flow (1.1 ml/min)	0.92
Wave length (328nm)	0.42
Wave length (348nm)	0.33
Organic phase composition (-2%)	0.69
Organic phase composition (+2%)	0.75
Column temperature (-5ºC)	0.81
Column temperature (+5º C)	1.02

Stability of AXT solution

Stability of the drug was studied at different conditions for quality control (QC) of samples. The drug was monitoring HPLC measured by every 30 min up to 24h. The samples were estimated with freshly analyzed QC samples, no difference was found in accuracy and precision during the analysis.

Analysis of Pharmaceutical formulation

The twenty tablets of Inlyta®, which label claim 5.0 mg of Axitinib, were weighted and ground manually to a fine powder using mortar and pestle. The weight equivalents to 5.0 mg of the axitinib powder transferred in a 25 ml volumetric flask added 15 ml of diluent, mixture was sonicated for 15min, volume was making up to mark with diluent and the solution was filtered using 0.45 nylon filters. Then 5.0 ml of this filtrate was transferred into a 10 ml volumetric flask and made up to 10 ml using diluents, to give a final concentration 100 µg/ml. All the measurements and chromatographic preparations were made at the room temperature. The assay is 100.15% results were shown in (Table 6) and the chromatogram of the formulation was shown in Figure 4.

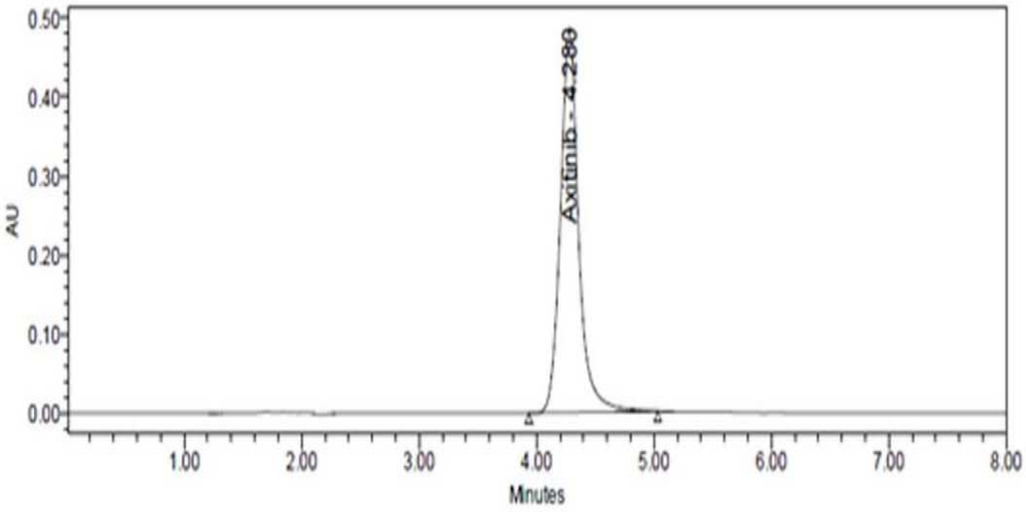


Figure 4: Chromatogram of pharmaceutical formulation (Inlyta®)

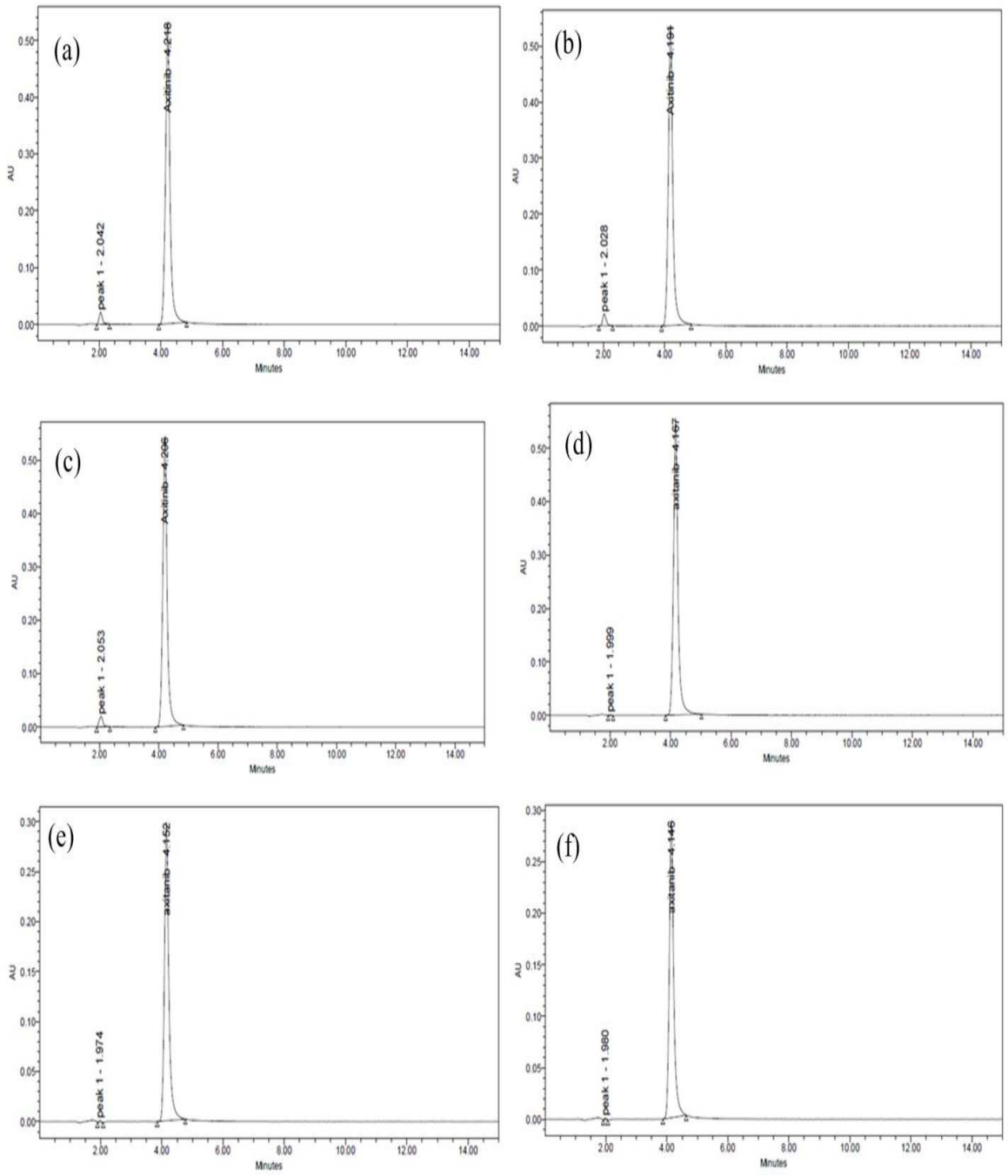
Table 6: Assay of Formulation (INLYTA®)

Pharmaceutical formulation	Label claim	Final concentration	Concentration found	%assay
INLYTA® tablets (Batchno:JP6874)	5.0 mg	100 µg/ ml	5.01	100.15

RESULTS AND DISCUSSION

Development and optimization of chromatographic conditions were executed to obtain the good peak shape, resolution, peak parameters (Retention time, tailing factor and theoretical plates) of the main peak. The average retention time was 4.28 ± 0.42 min, the overall time of analysis is 8.0 min and tailing factor and numbers of theoretical plates for axitinib were found to be 1.16 and 4149.2. The method was linear in the range of 25-250 µg/ml for axitinib with correlation coefficient was 0.999. Precision determined as intraday and interday variation for axitinib, %RSD were 0.55 and 0.45 which indicates that method was precise, with the %RSD was less than 2.0%, so method was specific, no interference observed when the drug was evaluated in the presence of excipients and forced degradation study [Figure 5]. Accuracy was determined by % recovery of drug from formulation were found to be 99.65, 99.37 and 99.87 and the mean recoveries were found to 99.63 and RSD 0.93% which indicates the method was accurate. The Limit of detection and quantification were found to be the 0.62 and 1.88 µg/ml. The selectivity of the method was estimated by the absence of any interference peaks from the drug retention time and method was applied for commercial formulations it found to be 100.15% which indicating the good compliance with the label claim.

Degradation behavior of AXT studied under the various stress conditions. The AXT was stable in neural, thermal and photolytic conditions percentage of drug degradations were below 1.0%. Acidic degradation was observed 1.60% at retention time 2.04 at 0.5 N HCl [Figure 5(a)], while for 1.0N, % of degradation was 3.49 at retention time 2.03 [Figure 5(b)]. Moreover, for 2.0 N the % of degradation was 5.31 at retention time is 2.053[Figure 5(c)]. Alkaline degradation was 2.41 at retention time 2.01 at 0.5 N [Figure 5(d)], while for 1.0N, percentage of degradation was 2.74 at retention time 2.03 [Figure 5(e)]. Moreover, for 2.0 N, the percentage of degradation was 3.69 at retention time 2.03[Figure 5(f)]. Oxidation degradation peak at retention time 2.29 with % of degradation was 6.50[Figure 5(g)]. Thermal degradation was 0.51% [Figure 5(h)] photolytic condition of degradation was 0.55% [Figure 5(i)] neural degradation was 0.24% [Figure 5(j)]. Results of stress degradation studies of AXT under different conditions are Table 7. The Statistical method used for compare results obtained by HPLC method and Reference method of Axitinib in Table 8.


(a)
Axitinib - 4.218
peak 1 - 2.042
AU
Minutes
(b)
Axitinib - 4.191
peak 1 - 2.028
AU
Minutes
(c)
Axitinib - 4.206
peak 1 - 2.053
AU
Minutes
(d)
axitanib - 4.167
peak 1 - 1.999
AU
Minutes
(e)
axitanib - 4.162
peak 1 - 1.974
AU
Minutes
(f)
axitanib - 4.146
peak 1 - 1.980
AU
Minutes

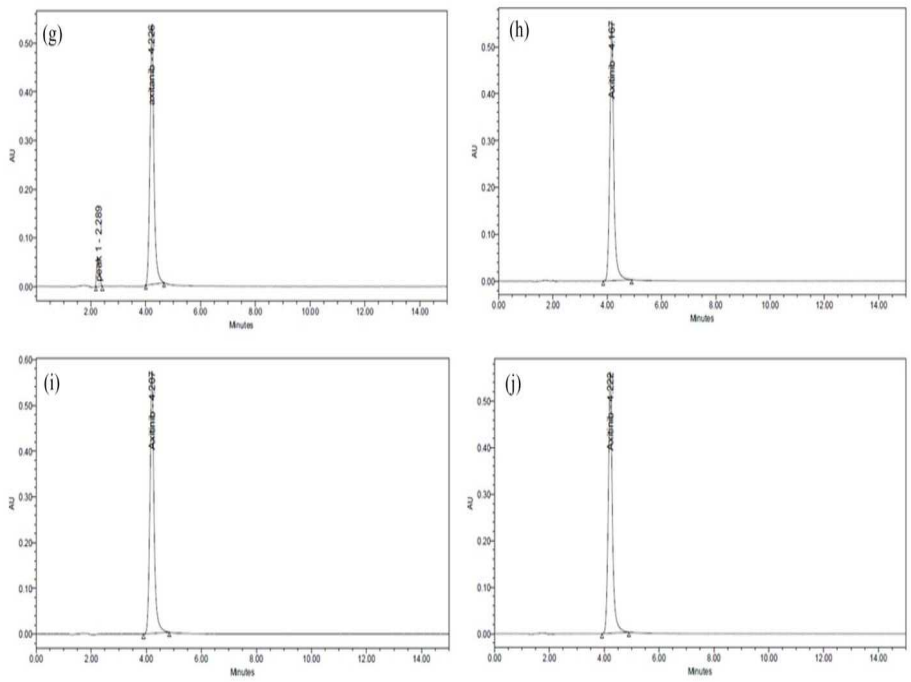


Figure 5: Chromatogram of (a) acid degradation 0.5 N HCl, (b) 1.0 N HCl (c) 2.0 N HCl (heated for 6 h at 60ºC), base degradation (d) 0.5 N NaOH, (e) 1.0 N NaOH (f) 2.0 N NaOH (heated for 6 h at 60ºC), (g) Chromatogram of H_2O_2 degradation (30 % v/v, heated for 6 h at 60ºC) (h) Thermal (heated for 6 h at 105°C) (i) Photochemical degradation (Direct sun light for 4 days) (j) Chromatogram of neutral degradation (heated for 6 h at 60ºC) treated Axitinib100 µg/ml

Table 7: Summary of stress degradation studies of Axitinib under different conditions

	Axitinib						
Stress conditions	**Degradant RRT**	**Asymmetry**	**Resolution**	**Purity Angle**	**Purity Threshold**	**% Drug Degraded**	**% Drug Remained**
Acid							
0.5N HCl, 60ºC, 6 h	0.48	1.2	9.3	0.073	0.306	1.60	98.40
1.0N HCl, 60ºC, 6 h	0.48	1.2	9.1	0.071	0.288	3.49	96.51
2.0 N HCl, 60ºC, 6 h	0.49	1.2	8.0	0.063	0.287	5.31	94.69
Neutral							
H_2O, 60ºC, 6 h	-	1.2	-	0.079	0.288	0.51	99.49
Base							
0.5N NaOH, 60ºC, 6 h	0.48	1.3	10.9	0.083	0.324	2.41	97.59
1.0N NaOH, 60ºC, 6 h	0.48	1.2	11.0	0.092	0.383	2.74	97.26
2.0N NaOH, 60ºC, 6 h	0.48	1.3	11.3	0.098	0.346	3.69	96.31
Oxidation							
30% H_2O_2 at room temperature for 6 h	0.54	1.2	9.3	0.094	0.389	6.50	93.50
Photolytic							
UV light 7 days	-	1.1	-	0.075	0.286	0.55	99.45
Thermal							
Dry heat, 105ºC, 6 h	-	1.23	-	0.078	0.284	0.24	99.74

Table 8: Statistical method compares results obtained by HPLC method and the Reference method of Axitinib and in pure form

Items	**HPLC method[1]**	**Reference method[8]**
Mean	100.15	99.83
%RSD	0.48	1.63
Variance	0.34	0.63
N	6	4
Student's t-test	0.011(1.86)[2]	
F-test	2.34(5.41)[2]	

[1] *RP-HPLC using an isocratic mixture of Potassium phosphate: acetonitrile at a flow rate of 1.0 ml /min and DAD detection at 338 nm.*
[2] *Figure between parentheses represent the corresponding tabulated values of t and F at P=0.05.*

CONCLUSION

The proposed RP-HPLC with DAD method provides a simple, accurate and reproducible method for the estimation of Axitinib and its degradant products. The developed method has stability indicating power to separate all the degradation products from Axitinib and stable under thermal, photolytic and neutral conditions. The method has been found best other than few methods reported, because cost effective, time saving, less solvent use, lack of extraction procedure and minimal elution time for both drug and degradant products within 8.0 min. The method was executing to the determination of drug in the pure and Pharmaceutical formulation without any interference from diluent and formulation excipients. Hence, this method can find many applications in pharmaceuticals for quantification and identification of degradants in quality control laboratories, stability studies in bulk and formulations.

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REFERENCES

[1] DD Hu-Lowe; HY Zou; ML Grazzini; ME Hallin; GR Wickman; K Amundson; SL Bender, *Clin Cancer Res*, **2008**,14(22),7272-7283.
[2] BI Rini; G Wilding; G Hudes; WM Stadler; S Kim; J Tarazi; JP Dutcher, *J. Clin. Oncol*, **2009**, 27(27),4462-4468.
[3] M McTigue; BW Murray; JH Chen; YL Deng; J Solowiej; RS Kania, *Proc Natl Acad Sci*, **2012**,109(45),18281-18289.
[4] Rixe; RM Bukowski, MD Michaelson, G Wilding; GR Hudes; O Bolte; BI Rini, *Lancet Oncol*, **2007**, 8(11),975-984.
[5] http://wwwaccessdatafdagov/drugsatfda_docs/label/2012/202324lblpdf browsed on 25th June **2015**.
[6] RW Sparidans; D Iusuf; AH Schinkel; JHM Schellens; JH Beijinen, *J. Chromatogr. B*, **2009**, 877(32),4090-4096.
[7] MD Likar ; G Cheng; N Mahajan; Z Zhang, *J Pharm Biomed Anal*, **2011**, 55(3), 569-573.
[8] B Lakshmi; K Saraswathi and TV Reddy, *IJSID*, **2012**, 2,184-190.
[9] JY Choi; T Ramasamy; TH Tran; SK Ku; BS Shin; HG Choi; JO Kim, *J. Mater. Chem. B*, **2015**, 3(3), 408-416.
[10] MA Zientek ; TC Goosen; E Tseng; J Lin; JN Bauman; GS Walker; BJ Smith, *Drug Metab Dispos*, **2015**, 44(1),102-14.
[11] ICH, Harmonized Tripartite Guideline. Stability Testing of New Drug Substances and Products Q1A (R2). International Conference on Harmonization, IFPMA, Geneva, **2003**.
[12] ICH, Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonization, IFPMA, Geneva, **2005**.
[13] The United States Pharmacopoeia MD, **2003**, 26, 1151-1154.
[14] M Bakshi; S Singh, *J Pharm Biomed Anal*, 2**0**02, 28, 1011-1040.
[15] SS Abbas; HE Zaazaa; HAM Essam; MG El-Bardicy, *J. AOAC Int*, **2014**, 97(1), 78-85.
[16] M Bakshi; B Singh; A Singh; S Singh, *J Pharm Biomed Anal*, **2011**, 26(5-6), 891-897.