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Research

A New Rp-Hplc Analytical Method Development And Validation Of Axitinib In Bulk And Its Pharmaceutical.

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Abstract

A simple and selective LC method is described for the determination of AXITINIB dosage forms. Chromatographic separation was achieved on a Zodiac, C18 (250×4.6× 5µ) column using mobile phase consisting of a mixture of Triethylamine: Acetonitrile (50:50) with detection of 254nm. Linearity was observed in the range 15-45 µg /ml for AXITINIB (r^2 =0.997) for the amount of drug estimated by the proposed methods was in good agreement with the label claim.

Keywords: High performance liquid chromatography, method validation, Axitinib-determination,HPLC.

INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books.[1]

Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – Qualitative and Quantitative analysis.

High Performance Liquid Chromatography

Chromatography is the method of separation that finds applications in all branches of science. It was first invented by Russian Botanist Mikhail Twsett. This technique was used separate various plant pigments like chlorophylls and xanthophylls by passing solutions of these compounds through a glass column packed with finely divided calcium carbonate. The separated species appeared as colored bands on the column hence the name of the process (Greek *chroma* meaning "color" and *graphein* meaning "writing").[3]

Chromatography is defined as a non- destructive procedure for resolving multi-component mixture of trace, minor, or major constituents into its individual fractions. In chromatography, the sample is dissolved in the mobile phase which may be a gas, liquid, or a supercritical fluid. The principle involved in HPLC is that when a mixture containing different compounds is introduced into the mobile phase and allowed to flow over a stationary phase, the individual compounds travel at different speeds and get separated based on the relative affinities to the stationary phase and the mobile phase. The compounds are separated based on the polarity of the stationary phase and the mobile phase.

Instrumentation of HPLC

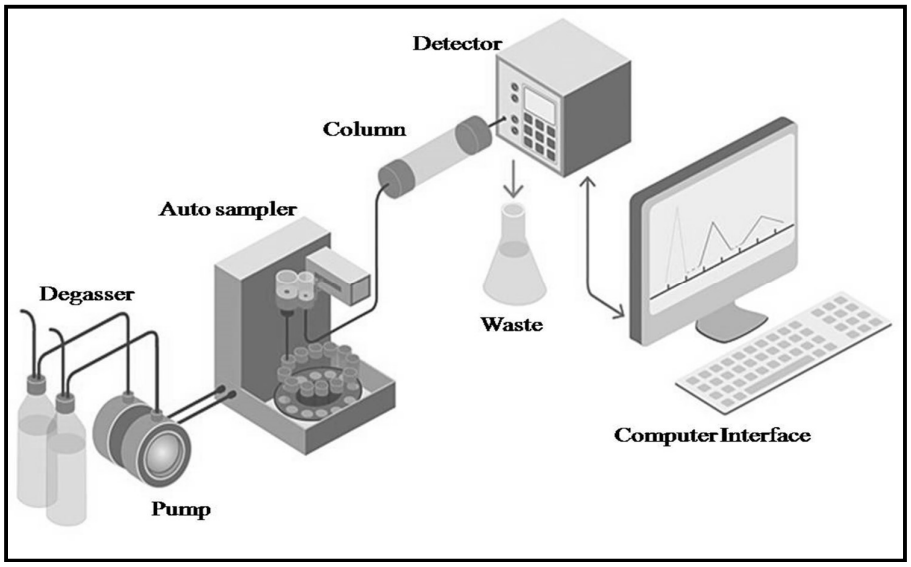


Fig1: Schematic diagram of instrumentation of HPLC.[18]

MATERIALS AND METHODS

Table 1: Instruments used

UV-Visible Spectrophotometer	Nicolet evolution 100
HPLC	Shimadzu(LC 20 AT VP)
HPLC	Agilent 1200 series
Ultra sonicator	Citizen, Digital Ultrasonic Cleaner
pH meter	Global digital
Electronic balance	Shimadzu
Syringe	Hamilton
HPLC Column	INERTSILcolumn,C18(150x4.6 ID) 5µm

Table 2: Reagents used

Water	HPLC Grade
Methanol	HPLC Grade
Potassium Dihydrogenortho Phosphate	AR Grade
Acetonitrile	HPLC Grade
Ammonium acetate	AR Grade
Tetra Hydro Furan	AR Grade

Table 3: Drug used

Axitinib	Gift Samples obtained from Chandra labs, Hyd.
INLYTA(5mg)	Obtained from local pharmacy

Mobile Phase

A mixture of 50 volumes of Triethylamine bufferpH 3.5 and50 volumes of Acetonitrile wasPrepared. The mobile phase was sonicated for 10min to remove gases.

RESULTS AND DISCUSSION

Determination of Working Wavelength (λmax)

In estimation of drug, wavelength is used.

Results

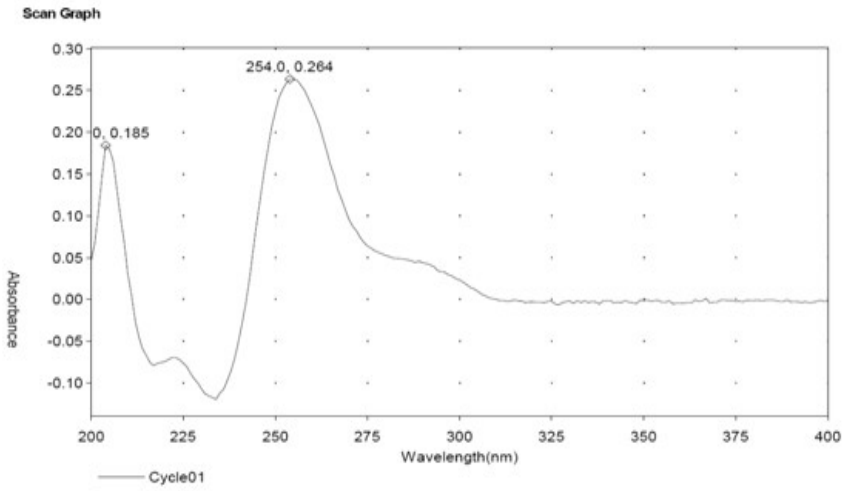


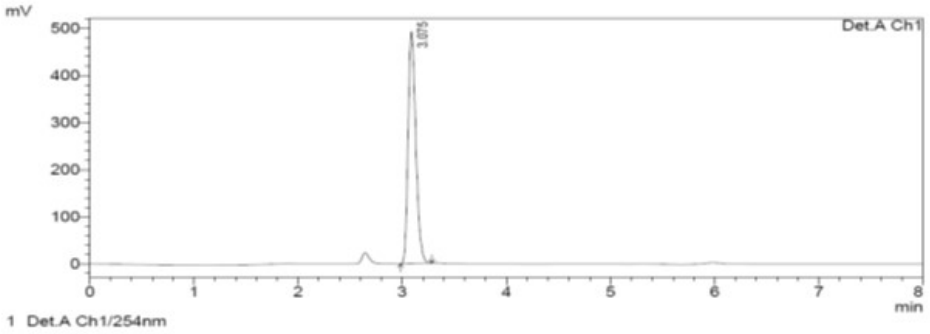
Fig 2: UV-VIS spectrum of AXITINIB.

Observation: λ_{max} was found to be254nm for AXITINIB.

METHOD DEVELOPMENT OF AXITINIB

Optimized Trail Chromatographic conditions

Mobile phase: TEA+ACN
Ratio : 50:50
Column : Zodiac, C18 (250×4.6× 5µ)
Wavelength : 254 nm
Flow rate : 1ml/min
pH : 3.5



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met
1	3.075	2562136	493044	45792.102
Total		2562136	493044	

Area %	Tailing Factor	Resolution
100.000	1.351	0.000
100.000		

Fig 3: Chromatogram of AXITINIB by using mobile phase.

Assay

Table 4: **Assay Results**

AXITINIB		
	Standard Area	**Sample Area**
Injection-1	2526136	2517760
Injection-2	2515827	2527548
Injection-3	2510709	2516884
Injection-4	2525250	2495644
Injection-5	2523113	2497496
Injection-6	2526637	2507449
Average Area	2521278.67	2510463.5
Assay(%purity)	99.5710444	

Validations

System Suitability and System Precision

Table 5: System Suitability

Name of the Standard	**AXITINIB**	**Tailing factor**	**Plate count**
Standard-01	2522232	1.36	46522.77
Standard-02	2516212	1.34	46373.68
Standard-03	2515844	1.34	46321.98
Standard-04	2522917	1.36	46512.29
Standard-05	2518764	1.33	46321.48
Average	2519193.80	-	-
%RSD	0.1	-	-

System Precision results

Table 6: System Precision results

Name of the Standard	**AXITINIB**
Standard-01	2522232
Standard-02	2516212
Standard-03	2515844
Standard-04	2522917
Standard-05	2518764

Standard-06	2515844
Average	2517916
%RSD	0.1

Specificity

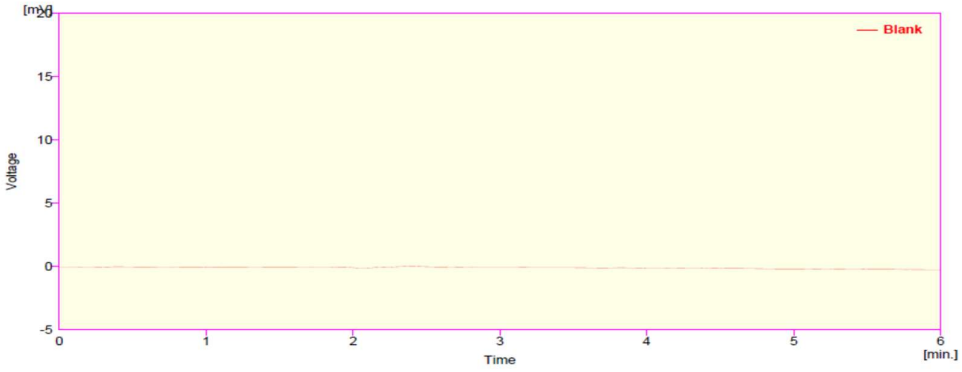
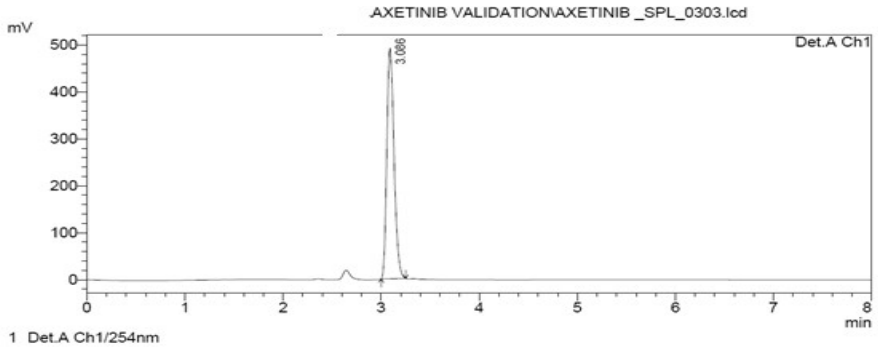


Fig 4: Blank chromatogram for specificity by using mobile phase



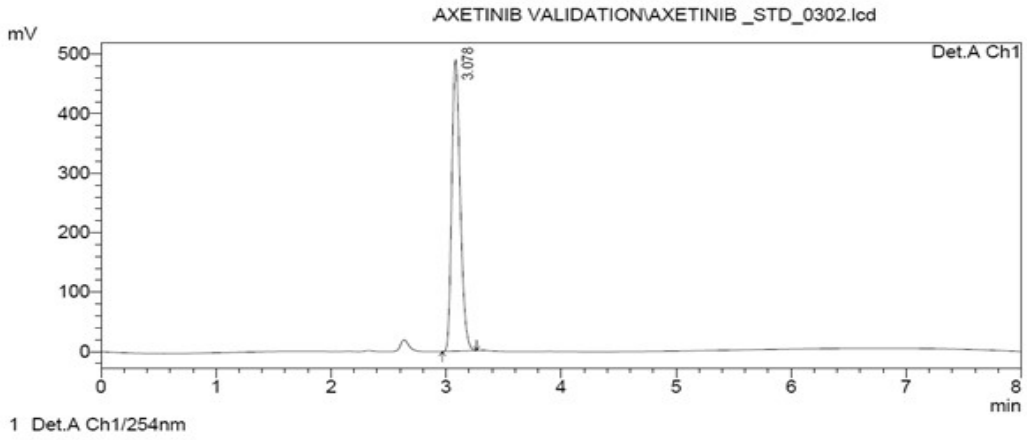
PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met
1	3.086	2504655	491726	46086.712
Total		2504655	491726	

Area %	Tailing Factor	Resolution
100.000	1.350	0.000
100.000		

Fig 5: Chromatogram for specificity of AXITINIB sample



PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met
1	3.078	2508561	490428	45711.541
Total		2508561	490428	

Area %	Tailing Factor	Resolution
100.000	1.353	0.000
100.000		

Fig 6: Chromatogram for Specificity of AXITINIB standard

Linearity and range

Table 7: Linearity Preparations

Preparations	Volume from standard stock	Volume made up in ml (with mobile	Concentration of
Preparation 1	1.5	10	15
Preparation 2	2.25	10	22.5
Preparation 3	3	10	30
Preparation 4	3.75	10	37.5
Preparation 5	4.5	10	45

Table 8:linearity of AXITINIB

S.No.	Conc.(µg/ml)	Area
1	15	1170177
2	22.5	1697912
3	30	2407163
4	37.5	2834924
5	45	3306552

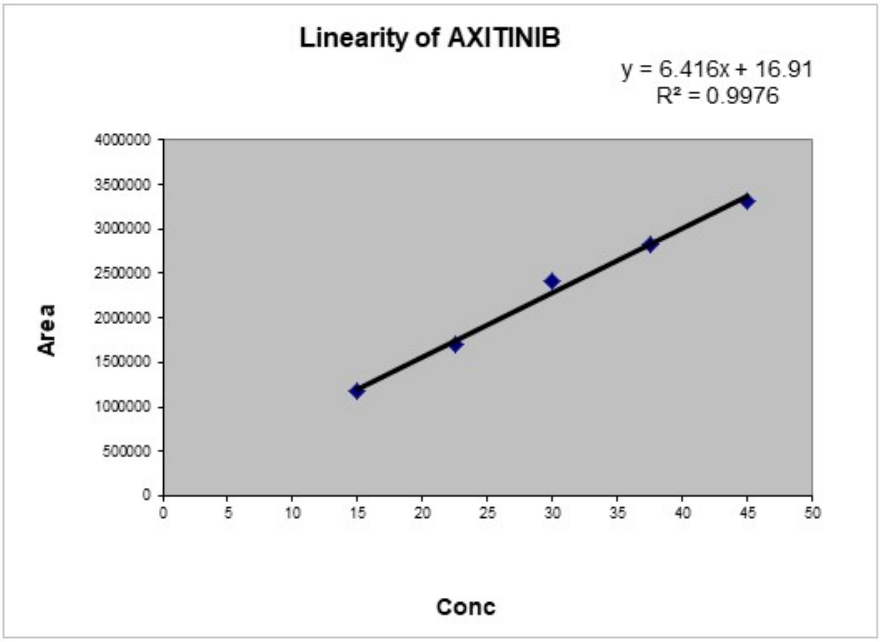


Fig 7: Linearity graph of AXITINIB.

Accuracy

Table 9: **Recovery results for AXITINIB**

Recovery level	Accuracy AXITINIB			
	Amount taken(mcg/ml)	Area	%Recovery	Average % Recovery
50%	15	1176833	100.66	
	15	1178517		
	15	1178517		
100%	30	2490174	101.54	
	30	2426700		100.5
	30	2415495		
150%	45	3292362	99.55	
	45	3285307		
	45	3297269		

The % recovery of AXITINIB should lie between 90% and 110%.

Method Precision

Table 10: Results for Method precision of AXITINIB

AXITINIB		
S.No.	**Rt**	**Area**
1	3.107	2073796
2	3.025	2036834
3	3.085	2078955
4	3.078	2075109
5	3.098	2063159
6	3.079	2075519
avg	3.07867	2067229
Stdev	0.02863	15823.2
%RSD	0.92983	0.76543

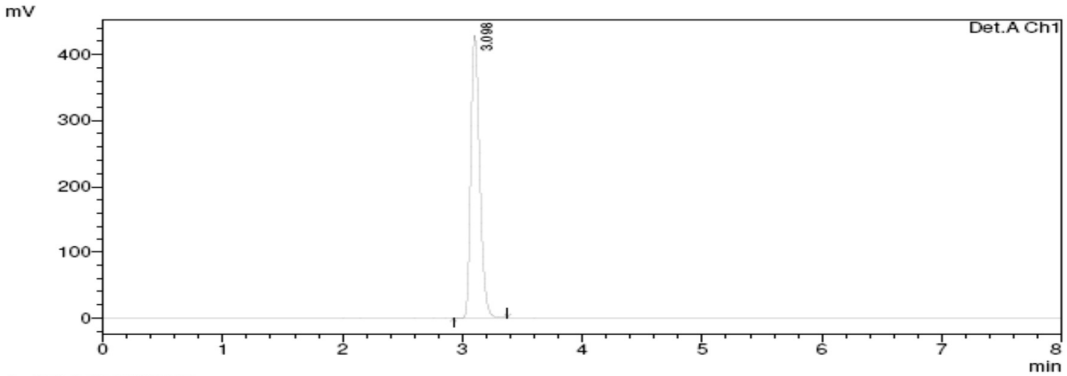
Robustness

Table 11:Results for Robustness of AXITINIB

Name of the Parameter	Theoretical Plates	Tailing factor
Low Column Oven Temperature(25°C)	53783.90	1.33
High Column Oven Temperature(35°C)	46799.55	1.35
Lower Wavelength(252nm)	46084.43	1.35
Higher Wavelength(254nm)	46799.55	1.35

Forced degradation study

Thermal degradation



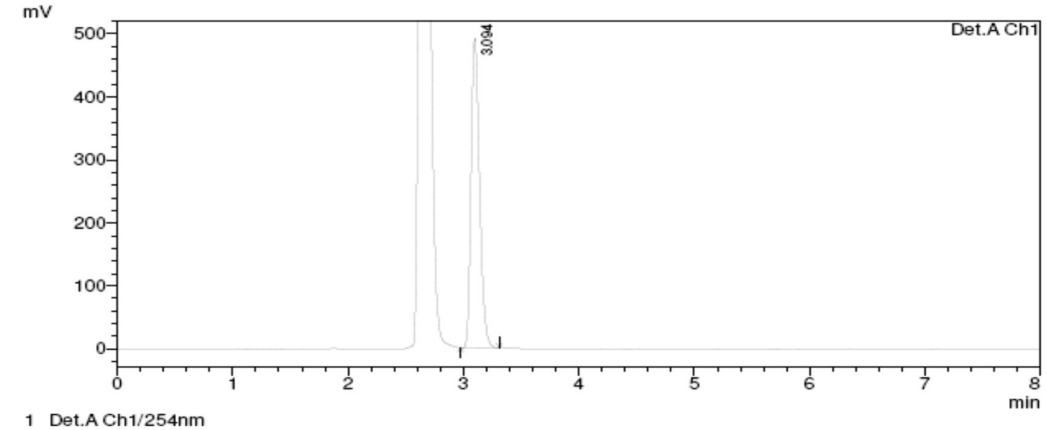
PeakTable

tector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met	Area %	Tailing Factor
1	3.098	2513365	428441	45837.367	100.000	1.362
Total		2513365	428441		100.000	

Fig 8: Chromatogram of Thermal Sample(105°C/72Hrs)

Photolytic degradation



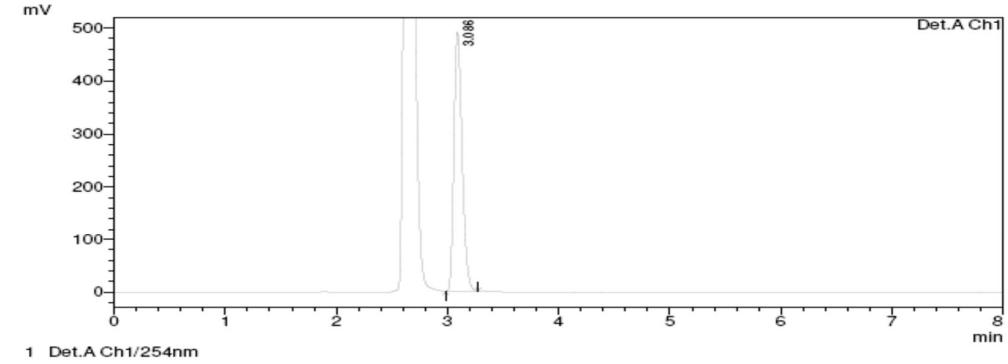
PeakTable

tector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met	Area %	Tailing Factor
1	3.094	2512791	490800	46010.652	100.000	1.352
Total		2512791	490800		100.000	

Fig 9: Chromatogram of Photolytic Sample(1.2mil LUX hrs)

Acidic degradation



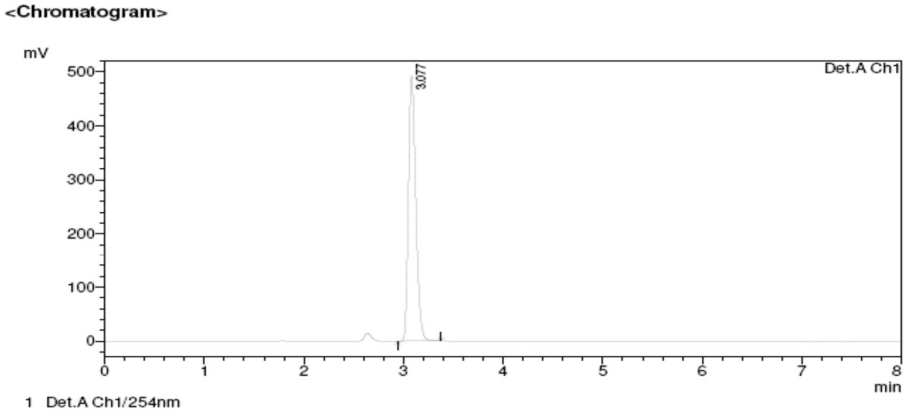
PeakTable

tector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/mete	Area %	Tailing Factor
1	3.086	2513105	490760	45819.321	100.000	1.354
Total		2513105	490760		100.000	

Fig 10: Chromatogram of Acid Sample preparation(5N HCl /4Hrs/60°C)

Alkaline degradation



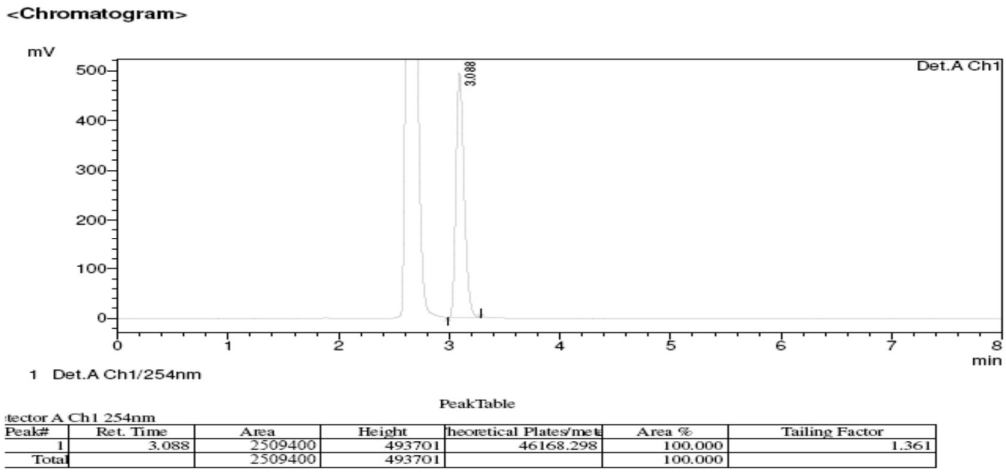
PeakTable

tector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/mete	Area %	Tailing Factor
1	3.077	2507427	492761	45772.461	100.000	1.356
Total		2507427	492761		100.000	

Fig 11: Chromatogram of Base Sample preparations(5N HCl /4Hrs/60°C)

Peroxide degradation



PeakTable

tector A Ch1 254nm

Peak#	Ret. Time	Area	Height	heoretical Plates/met	Area %	Tailing Factor
1	3.088	2509400	493701	46168.298	100.000	1.361
Total		2509400	493701		100.000	

Fig 12: Chromatogram of Peroxide Sample preparation(30%H2O2/Benchtop/4hrs)

Table 12: Force degradation Results of AXITINIB

	AXITINIB			
Injection	**Condition**	**Area**	**% Assay**	**% Degraded**
1	Thermal	2513365	99.387	0.183
2	Photolytic	2512791	99.364	0.206
3	Acid Hydrolysis	2513105	99.377	0.193
4	Base Hydrolysis	2507427	99.152	0.418
5	Peroxide Hydrolysis	2509400	99.230	0.340

The % Degraded for Axitinib from these stability methodsshouldbe not more than 1.0 %.

DISCUSSION

A simple and selective LC method is described for the determination of AXITINIB dosage forms. Chromatographic separation was achieved on a Zodiac, C18 (250×4.6× 5µ)column using mobile phase consisting of a mixture of Triethylamine: Acetonitrile(50:50)with detection of 254nm. Linearity was observed in the range 15-45 µg /ml for AXITINIB(r^2 =0.997)for the amount of drug estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of AXITINIB was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

REFERENCES

1. Inlyta (axitinib) dosing, indications, interactions, adverse effects, and more". Medscape Reference. WebMD. Retrieved 25 January 2014.https://reference.medscape.com/drug/inlyta-axitinib-999715
2. Wilmes LJ, Pallavicini MG, Fleming LM, Gibbs J, Wang D, Li KL, Partridge SC, Henry RG, Shalinsky DR, Hu-Lowe D, Park JW. AG-013736, a novel inhibitor of VEGF receptor tyrosine kinases, inhibits

breast cancer growth and decreases vascular permeability as detected by dynamic contrast-enhanced magnetic resonance imaging. Magnetic resonance imaging. 2007 Apr 1;25(3):319-27.https://www.sciencedirect.com/science/article/pii/S0730725X06003079

3. Rini B, Rixe O, Bukowski R, Michaelson MD, Wilding G, Hudes G, Bolte O, Steinfeldt H, Reich SD, Motzer R. AG-013736, a multi-target tyrosine kinase receptor inhibitor, demonstrates anti-tumor activity in a Phase 2 study of cytokine-refractory, metastatic renal cell cancer (RCC). Journal of clinical oncology. 2005 Jun 1;23(16_suppl):4509. https://ascopubs.org/doi/abs/10.1200/jco.2005.23.16_suppl.4509
4. Rugo HS, Herbst RS, Liu G, Park JW, Kies MS, Steinfeldt HM, Pithavala YK, Reich SD, Freddo JL, Wilding G. Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. Journal of Clinical Oncology. 2005 Aug 20;23(24):5474-83. doi:10.1200/JCO.2005.04.192. PMID 16027439.https://ascopubs.org/doi/abs/10.1200/JCO.2005.04.192
5. Tyler T. Axitinib: newly approved for renal cell carcinoma. J AdvPractOncol. 2012 Sep;3(5):333-5. doi: 10.6004/jadpro.2012.3.5.7. PMID: 25031963; PMCID: PMC4093354.
6. John Fauber; Elbert Chu (Oct 27, 2014). The Slippery Slope: Is a Surrogate Endpoint Evidence of Efficacy?. *Milwaukee Journal Sentinel/MedPage Today*.
7. INLYTA (axitinib) tablet, film coated [Pfizer Laboratories Div Pfizer Inc]. DailyMed. Pfizer Laboratories Div Pfizer Inc. September 2013. Retrieved 25 January 2014.