CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1210043

Available online at: http://www.iajps.com Research Article

A NEW METHOD DEVELOPMENT AND VALIDATION OF AXITINIB BULK AND PHARMACEUTICAL DOSAGE FORM BY USINGUV-VISIBLE SPECTROSCOPY AS PER ICH GUIDELINES

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Abstract:

The objective of the present work was to develop a simple, efficient and reproducible spectrophotometric method for the quantitative estimation of Axitinib drug in its active pharmaceutical ingredient (API) form. The developed UV-Visible spectrophotometric method for the quantitative estimation of drug –Axitinib measurement of absorption at a wavelength maximum (λmax) of 260 nm using methanol as diluents. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of Axitinib in pharmaceutical dosage forms.The linearity dynamic range 10-70 µg/ml and effective mean percentage recoveries were 102.5% and LOQ, LOD values of Axitinib were found to be 1.62 and 5.04(µg/ml)

Key Words: ***Axitinib, Method Development, Validation, UV-Visible spectrophotometry.***

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Please cite this article in press G. Koteshwar Rao et al., ***A New Method Development and Validation of Axitinib Bulk and Pharmaceutical Dosage Form by Using UV-Visible Spectroscopy as Per Ich Guidelines,*** *Indo Am. J. P. Sci, 2018; 05(03).*

INTRODUCTION:

Axitinibis anAnti-cancer (Anti neoplastic), molecular formula$C_{22}H_{18}N_4OS$, IUPAC name: N-methyl-2-({3-[(E)-2-(pyridin-2-yl) ethynyl]-2H-indazol-6yl} sulfanyl) benzene-1-carboximidic acid.Axitinib is a second-generation tyrosine kinase inhibitor. It selectively inhibits vascular endothelial growth factor receptors (VEGFR-1, VEGFR-2, and VEGFR-3) thus blocking angiogenesis, tumour growth and metastases.

Axitinib has been reported to be 50-450 times more potent than first generation VEGFR inhibitors. According to literature review [12-17] there are very few method reported for the determination of Axitinibin different Instrumental techniques.

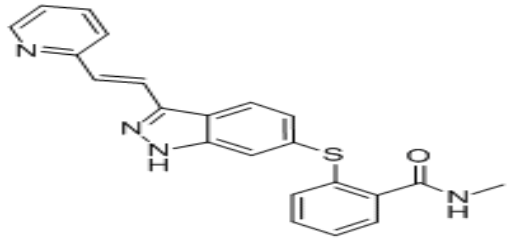

Fig. 1: Shows structure of Axitinib

EXPERIMENTAL SECTION:

Standard drugs:

Axitinibwas procured from the INLYTA Pharma.

Chemicals and reagents:

Methanol (FINER chemical LTD), Purified water (Rankem chemicals).

Instruments:

UV (SHIMADZU 1601), Sonicator (Analytical technologies).

Determination of absorption maxima by UV/Visible Spectrophotometry:

Accurately weigh 10 mg of drug in to 10 ml volumetric flask. To this add 10 ml of diluent Methanol and sonicate it and further make up the volume with diluent. From this take 1 ml and make up to10ml. The solutions were scanned in the range of 200-400 nm in 1cm cell against blank.

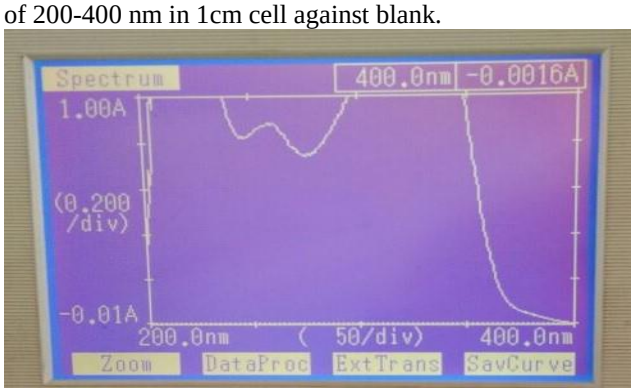

Fig.2: Shows UV spectrum of Axitinib

Preparation of mobile phase:

Accurately measured 100 ml of Methanol, were degassed in an ultrasonic water bathfor 10minutes and then filtered through 0.45 µ nylon filter under vacuum filtration.

Diluent:

Mobile phase is used as diluent

Standard preparation:

Accurately weigh 10 mg of Axitinib and transfer in to 10ml volumetric flask. Add about 10ml of solventmixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 1ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

Sample preparation:

Accurately weigh 10 mg of Axitinib powder and transfer in to 10ml volumetric flask. Add about 10ml of solventmixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 1ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

Optimized chromatographic conditions:

Wavelength - 260nm

Solvent - methanol

Method validation:

The following parameters were considered for the analytical method validation of Axitinib in bulk form & tablet dosage form.

System Suitability:

Chromatograph the standard preparations (6 replicate concentrations) and measure the absorbance evaluate the system suitability parameters as directed.

Accuracy:

For accuracy determination, three different concentrations were prepared separately 8%, 100% and 120%for the concentrations of absorbance values are recorded.

Precision:

The standard solution was placed into cuvettes for six times and measured for all six concentrations absorbance values by using max in UV. The %RSD forthe area of six replicate concentrations was found to be within the specified limits.

Ruggedness:

As part of the Ruggedness, deliberate variations in method parameters and provides an indication of its reliability during normal usage. Wavelength was varied between plus or minus to. the solutions were made in triplicates and were analyzed the %RSD is determined.

Linearity and range:

Linearity of the analytical method for assay by placing the linearity solutions prepared in the range of 10µg to 70µgof test concentration, into the cuvettes, covering minimum 7 different concentrations.

RESULTS AND DISCUSSION:

Standard preparation

Accurately weigh 10 mg of Axitinib and transfer in to 10ml volumetric flask. Add about 10ml of solventmixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Transfer 1ml of above solution in to a 10ml volumetric flask and make up the volume with diluent.

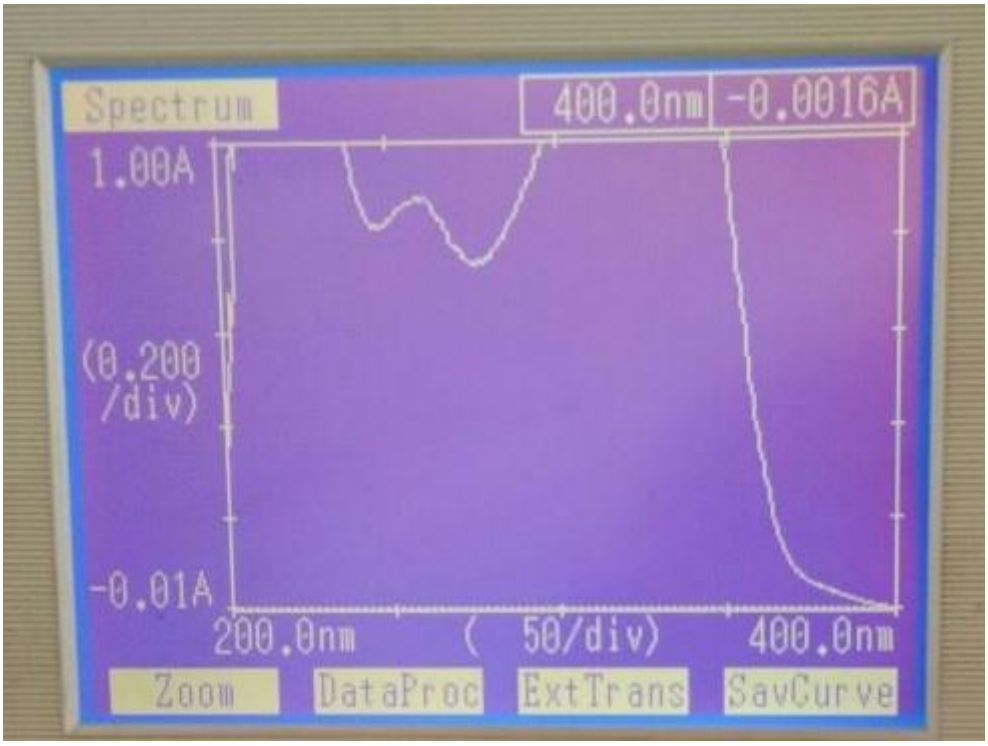

Fig. 3: Shows UV absorption spectrum of Axitinib standard

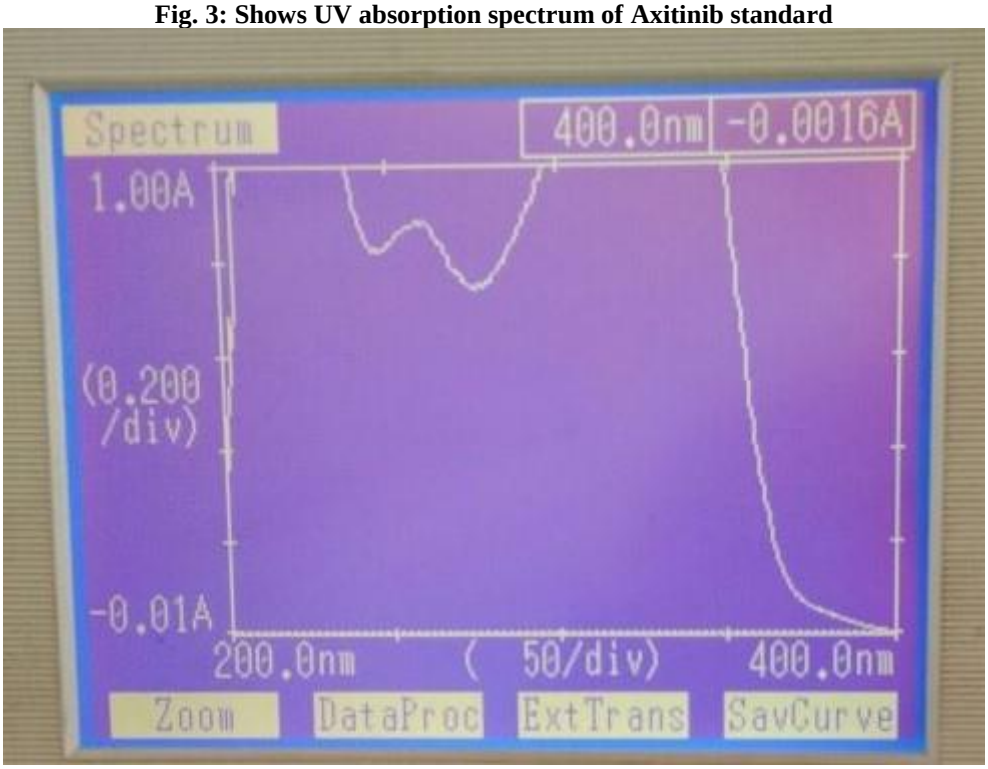

Fig. 4: Shows UV absorption spectrum of Axitinib sample Validation

Accuracy:
Average recoveries of Axitinib are 102.85%, 105.70%, 111.42%, at 80%, 100% & 120% concentrations levelrespectively. The percentage recoveries of the drug are within the limits 98-102%. So, the method is accurate, accuracy data for Axitinib are presented in;

Table 1: Shows Accuracy results of Axitinib

Concentration level	Amount added (mg)	%recovery	Average % recovery
80%	8mg 8mg 8mg	97.142% 102.85% 108.57%	102.85%
100%	10mg 10mg 10mg	102.85% 101.42% 102.85%	102.37%
120%	12mg 12mg 12mg	101.42% 101.42% 101.42%	101.42%

RESULTS:
The accuracy for the average of triplicate in each concentration samples are within the limit.

Table 2: Shows % Recovery of Axitinib

Amount added (mg)	Amount found(mg)	Average % recovery
10mg	10.237mg	102.370%

Precision:
Precision are summarized in **Table No: 3**, respectively. The %RSD values for Precession was less than 2.0%, whichindicates that the proposed method is precise.

Table 3: Shows Precision Results of Axitinib

Concentration (µg/ml)	Absorbance of Axitinib
10	0.361
10	0.343
10	0.361
10	0.361
10	0.361
10	0.361
Mean	**0.3581**
SD	**0.000054**
%RSD	**0.015**

Linearity:
The response was found linear over a concentration range of 10-70µg/mL of Axitinib. Thecorrelation co-efficient were found to be 0.9165 for Axitinib**.** So the method is linear, data is presented in

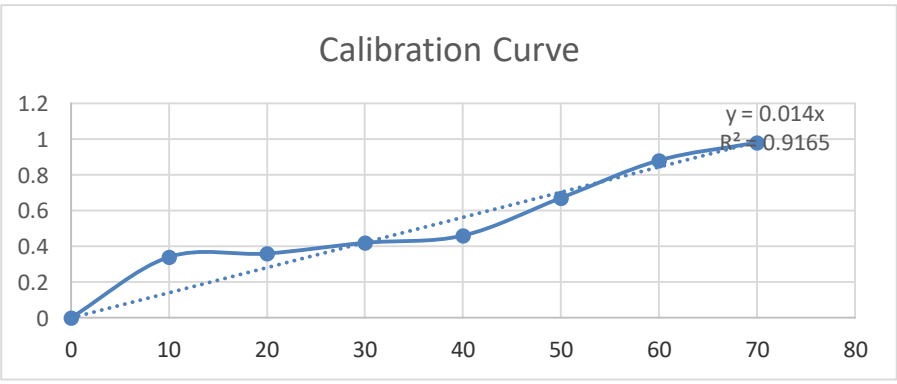

Table 4: Shows linearity results of Axitinib

S.no	Linerty level	Concentration	Area
1	I	10µg	0.34
2	II	20 µg	0.36
3	III	30 µg	0.42
4	IV	40 µg	0.46
5	V	50 µg	0.67
6	VI	60 µg	0.88
7	VII	70 µg	0.98
Correlation Coefficient			0.9165
Intercept			Y=0.014x+0.001
Slope			0.001

4) Ruggedness:

The Ruggedness of the method was determined by making slight changes in the experimental conditions such as change in the wavelength.

Table 5: Shows Results of Ruggedness

Analyst-1

S.no	Linerty level	Concentration	Area
1	I	10µg	0.361
2	II	20 µg	0.343
3	III	30 µg	0.3611
4	IV	40 µg	0.3613
5	V	50 µg	0.3612
6	VI	60 µg	0.3612
Correlation Coefficient			0.9165
Intercept			Y=0.014x+0.001
Slope			0.001

Analyst-2

S.no	Linerty level	Concentration	Area
1	I	10µg	0.361
2	II	20 µg	0.343
3	III	30 µg	0.3611
4	IV	40 µg	0.362
5	V	50 µg	0.364
6	VI	60 µg	0.367
Correlation Coefficient			0.9165
Intercept			Y=0.014x+0.001
Slope			0.001

Limit of Detection (LOD)&LOQ: The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected **,** The LOD are calculated from the calibration curve by formula LOD = 3.3 x SD/ b The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum levelat which the analyte can be quantified with acceptable accuracy and precision, The LOQ are calculated from the calibration curve by formula LOQ = 10 x SD/ b

Table 6: Shows LOD & LOQ results of Axitinib

Parameters	**Axitinib**
LOD	1.68µg/ml
LOQ	5.04µg/ml

VALIDATION PARAMETER RESULTS

Table 7: Shows summary of validation parameter Results

S.NO	**Parameter**	**Acceptance criteria**	**UV**
1	%recovery	92-103%	102.37%
2	Linearity range (µg/ml)	-	10-70(µg/ml)
3	Correlation Coefficent	NLT 0.999	0.9165
4	Precision	%RSD(NMT 2%)	0.015
5	Intermediate Precision	%RSD(NMT 2%)	0.18
6	Ruggedness	%RSD(NMT 2%)	0.10
7	LOD	-	1.68(µg/ml)
8	LOQ	-	5.04(µg/ml)

CONCLUSION:

In the present investigation, we are find out a simple, sensitive, precise and accurate UV-Visible spectroscopy method is developed for the quantitative estimation of axitinib in bulk drug and pharmaceutical dosage forms from the literature review.

This method is simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Axitinib is freely soluble in DMSO and ethanol, methanol and sparingly soluble in water.

This method can be used for the routine determination of axitinib in bulk drug and in Pharmaceutical dosage forms

ACKNOWLEDGEMENT:

The authors are grateful to the Management of Nalanda College of Pharmacy, Nalgonda for providing the facilities carry out the present research our work.

REFERENCES:

1. A.H. Beckett and J.B. Stenlake, Practical Pharmaceutical Chemistry, 4th Edn. Part II, CBS Publisher and Distributor, (2002), 1.
2. A. Douglas Skoog and M. Donald West, Principles of Instrumental Analysis, 2nd Edn. Saunders Golden Sunburst Series, (1980), 667.
3. G.H. Jeffery, J. Bassett, J. Mendham and R.C. Denney, Vogel's Text Book of Qualitative Analysis, 5th Edn. Longman Scientific & Technical Publication, (1988), 708.
4. P.D. Sethi, Quantitative Analysis of Pharmaceutical Formulations, 3rd Edn. CBS Publishers and Distributors, (1997), 51.
5. G.D Christian, Analytical Chemistry, 6th Edn. Wiley International, (2004), 604.
6. G.W. Ewing, Instrumental Methods of Chemical Analysis, 5th Edn. McGraw Hill International, (1985), 3.
7. P.R. Brown, Analytical Chemistry, (1990), Vol. 62, 995.
8. B.K. Sharma, Instrumental Methods of Chemical Analysis, Goel Publishers, (1999), 13.
9. "Product information inlyta (axitinib)"*(PDF)*. TGA eBusiness Services. Pfizer Australia Pty Ltd. 5 July 2013. Retrieved 25 January 2014.
10. Spano, JP; Chodkiewicz, C; Maurel, J; Wong, R; Wasan, H; Barone, C; Létourneau, R; Bajetta, E; Pithavala, Y; Bycott, P; Trask, P; Liau, K; Ricart, AD; Kim, S; Rixe, O (June 2008). "Efficacy of gemcitabine plus axitinib compared with gemcitabine alone in patients with advanced pancreatic cancer: an open-label randomised phase II study". Lancet. **371** (9630): *2101–2108.* PMID 18514303. doi:10.1016/S0140-6736(08)60661-3

11.B.Lakshmi, Prof.K.Saraswathi*, Prof. T.V.Reddy**RP-HPLC method development and validation for the analysis of Axitinib pharmaceutical dosage formsB. Lakshmi et al., IJSID, 2012; 2(1):184-190
12. Gorja Ashok, Sumantha Mondal Development and Validation of Stability indicating method for the estimation of Axitinib in tablet dosage forms by UPLC,Indian J.Pharm.Biol.Res.2017; 5(3):1-6
13. High-Speed Analysis of Sunitinib and Axitinib in Plasma Using Triple Quadrupole LC/MS/MS (LCMS-8050)www.shimadzu.com/an/
14. Yi Qu a,b, Naouel Gharbic, Xing Yuand, Jan Roger OlsenaPernille Blichere,Bjørn Dalhuse,f, Karl A. Brokstada,g,Biaoyang Linh Anne Margrete Øyana, Weidong Zhangd, Karl-Henning Kallanda,b,i,1, and Xisong Kea,1Axitinib blocks Wnt/β-catenin signaling and directs asymmetric cell division in cancer.
15. Manolis Vasileiadis, Constantinos C. Pantelides, Claire S. Adjiman, Prediction of the crystal structures of axitinib, a polymorphic pharmaceutical molecule
16. V. Ashok Chakravarthy1* and. B. V. Sailaja Method development and validation of uv-visible spectroscopic method for the estimation of assay of anticancer drugs-axitinib, bostunib,erlotinib hydrochloride, gefitinib and pemetrexed disodium drugs in api form .ejpmr, 2016,3(12), 609-624
17.Pfizer Products Inc. INLYTA® product monograph. Kirkland, Quebec; 30 July 2013.