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Analytical method development and validation for the simultaneous estimation of axitinib by using RP-HPLC technique

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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 c_{18} column using mobile phase consisting of a mixture of Triethylamine Buffer: 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.

Keywords: Axitinib, %RSD, range 15-45 µg /ml.

INTRODUCTION

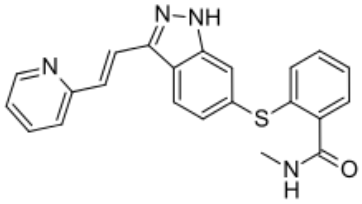
A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books. Pharmaceutical analysis is 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. Qualitative analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half life etc.

INTRODUCTION TO DRUG

Axitinib

Axitinib is an oral, potent, and selective inhibitor of vascular endothelial growth factor receptors (VEGFR) 1, 2, and 3. Axitinib is marketed under the name Inlyta®, and if one previous systemic therapy for kidney cell cancer has failed, axitinib is indicated.

Structure:



Iupac Name: N-methyl-2-({3-[(E)-2-(pyridin-2-yl)ethenyl]-2H-indazol-6-yl}sulfanyl)benzene-1-carboximidic acid
Molecular weight: 386.47
Chemical formula: $C_{22}H_{18}N_4OS$

AIM

To develop new RP HPLC method for the simultaneous estimation of Axitinib in pharmaceutical dosage form.

MATERIALS AND METHODS

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

Reagents used

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

Drug used

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

METHOD DEVELOPMENT AND VALIDATION

Introduction to Method Development

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Often a time lag exists from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias.

Method Development Using HPLC

In method development, an attempt to select the best chromatographic conditions like the best column, the best mobile phase, the detection wavelength etc. to be used for routine analysis of any drug is done. For the method development by HPLC method some information about the sample is very essential i.e. number of components present in the sample, pKa values of different components, UV-Visible Spectra of each analyte, solubility in different solvents, concentration range of each component, nature of sample etc.

Further Optimization

After the selection of a suitable method, mobile phase, column and detector, further optimization can be done to obtain a well developed method.

For better selectivity and sensitivity: Other stationary phases e.g. phenyl, CN etc. pH control with ion-forming compounds. Use of methanol or THF instead of acetonitrile. Detection at the absorption maximum of the substance. All factors which leads to narrower and higher peaks as gradient elution, smaller particle, micro bore columns.[35]

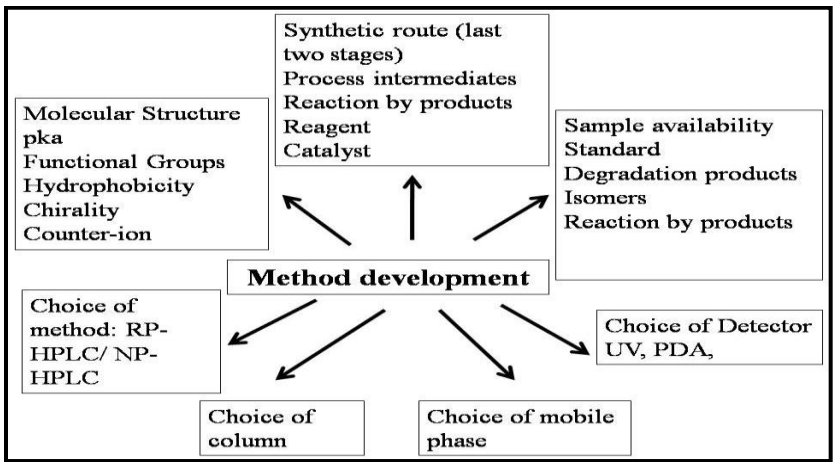


Fig 1: Outline of the process involved in method development

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 100 µg/ml solution of the drug in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank, and the absorption curve shows characteristic absorption maxima at 254 nm for AXITINIB. λ_{max} was found to be 254 nm for AXITINIB..

Assay

Preparation of samples for Assay

Preparation of standard solution

Weigh accurately 10 mg of AXITINIB in 100 ml of volumetric flask and dissolve in 100ml of mobile phase and make up the volume with mobile phase From above stock solution 30 µg/ml of AXITINIB is prepared by diluting 3ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

10 tablets (each tablet contains 5mg of AXITINIB was weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of AXITINIB (100µg/ml) were prepared by dissolving weight equivalent to 5 mg of AXITINIB and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 30µg/ml of AXITINIB was made by adding 3 ml of stock solution to 10 ml of mobile phase.

$$\%\ \text{Assay} = \frac{\text{AT}}{\text{AS}} \times \frac{\text{WS}}{\text{DS}} \times \frac{\text{DT}}{\text{WT}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where,

AS: Average peak area due to standard preparation, AT: Peak area due to assay preparation, WS: Weight of AXITINIB in mg. WT: Weight of sample in assay preparation, DT: Dilution of assay preparation.

VALIDATION

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of standard solution

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Linearity and range

Preparation of mixed standard solution

weigh accurately 100 mg of AXITINIB in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.

The relationship between the concentrations of AXITINIB should be linear in the specified range and the correlation should not be less than 0.99. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of AXITINIB is 0.991 is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analysed sample), the reference standards of the drugs were added at the level of 75%, 100%, 125%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analysed sample solution at three different levels 75%, 100% & 125%.

Precision

Method precision

Prepared sample preparations of AXITINIB as per test method and injected 6 times in to the column. The % Relative standard deviation of Assay preparations of AXITINIB should be not more than 2.0%. Test results for AXITINIB was showing that the %RSD of Assay results are within limits

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision. The system suitability should pass as per the test method at variable conditions.

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts. The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%. From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.

DISCUSSION

A simple and selective LC method is described for the determination of AXITINIB dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Triethylamine Buffer: 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 Ref WebMD.
2. Wilmes LJ, Pallavicini MG, Fleming LM, Gibbs J, Wang D, Li KL et al.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. Magn Reson Imaging. April 2007."AG;25(3):319-27. doi:10.1016/j.mri.2006.09.041, PMID 17371720.
3. Rixe O, Bukowski R, Michaelson MD, Wilding G, Hudes G, Bolte O et al. Jump up^ Rini, B. Agriculturists-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 ASCO Annual Meeting Proceedings. June 2005;23(16S):4509.
4. Rugo HS, Herbst RS, Liu G, Park JW, Kies MS, Steinfeldt HM et al.Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. J Clin Oncol. August 2005; Herbst;23(24):5474-83. doi: 10.1200/JCO.2005.04.192, PMID 16027439.
5. FDA approves Inlyta for advanced renal cell carcinoma". Drugs.com; January 27, 2012.
6. John Fauber; Elbert Chu. Milw J Sentinel/MedPage Today. October 27, 2014. "The Slippery Slope: Is a Surrogate Endpoint Evidence of Efficacy?"
7. "INLYTA (axitinib) tablet, film coated [Pfizer Laboratories Div Pfizer Inc]". DailyMed. Pfizer Laboratories Div Pfizer Inc. September 2013. [retrieved Jan 25 2014].
8. "Inlyta: EPAR – product information" (PDF). [Retrieved Jan 25 2014]. European Medicines Agency. Pfizer Ltd.; December 17 2013.
9. "Inlyta 1 mg 3mg, 5 mg & 7-mg film-coated tablets – Summary of Product Characteristics (SPC)". electronic Medicines Compendium. Pfizer Limited. December 5 2013. [Retrieved Jan 25 2014].
10. Product Information Inlyta (axitinib). Arkinson's Disease Foundation. TGA eBusiness Services. Pfizer Australia Pty. Ltd.; July 5 2013.