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Determination, Development and Validation of Method for Simultaneous AXITINIB Pharmaceutical Dosage form by a Reverse Phase HPLC

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Abstract

A simple and selective LC method is described for the determination of AXITINIB dosage forms. Chromatographic separation is observed on a c_{18} packing column using mobile phase with a mixture of TEA -Acetonitrile (50:50) with detected at 254nm. Linearity was observed in the range 15-45 µg /ml for AXITINIB (r^2 =0.997) for drug estimated by the proposed methods was in good agreement with the label claim. The proposed method was validated. The accuracy of the methods was assessed by recovery studies at three different levels. The current study indicated the absence of interference of commonly encountered pharmaceutical additives. The method was precise to be indicated by the repeatability analysis, by showing %RSD > 2. All statistical data proved the validity of the methods and used for routine analysis of pharmaceutical dosage form.

Keywords: *axitinib, RP-HPLC, validation.*

INTRODUCTION

A drug intended for internal or external use for 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.

Quantitative analysis techniques are mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained the characteristic [1, 2] movement of a substance through a defined medium under controlled conditions, electrical measurement or measurement of spectroscopic properties of the compound. 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.

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. Types of HPLC [15, 16] HPLC is classified into various types

Based on polarity of stationary and mobile phase

- Normal Phase Chromatography
- Reverse Phase Chromatography

Based on the principle of separation

- Adsorption Chromatography
- Partition Chromatography
- Ion Pair Chromatography
- Size Exclusion Chromatography
- Chiral Phase Chromatography

Based on elution technique

- Isocratic Elution
- Gradient Elution

Based on scale of operation

- Analytical HPLC
- Preparative HPLC

Based on the polarity of the stationary phase and the mobile phase, it is of two types:

Normal Phase (NP) HPLC

In this type, the stationary phase is polar, and the mobile phase is non-polar, polar compounds are retained for a longer period because of more affinity towards the stationary phase, hence non-polar compounds travel faster and are eluted first.

Reverse Phase (RP) HPLC

In this type, the stationary phase is non-polar, and the mobile phase is polar, non-polar compounds are retained for longer periods as they have more affinity towards the stationary phase. Hence, polar compounds travel faster and are eluted first [2]

Table 1: *Types of HPLC based on polarity of stationary phase and mobile phase.*

Types	Normal Phase	Reverse Phase
Stationary phase	Polar	Non polar
Mobile phase	Non polar	Polar
Compound eluted first	Non polar	Polar
Compound eluted last	Polar	Non polar

Instrumentation of HPLC

The main components of HPLC are as given below and are schematically represented

1. Solvent Reservoir
2. Solvent Delivery System (Pump)
3. Injection Port/ Auto sampler
4. Column
5. Detector
6. Data Acquisition system
7.

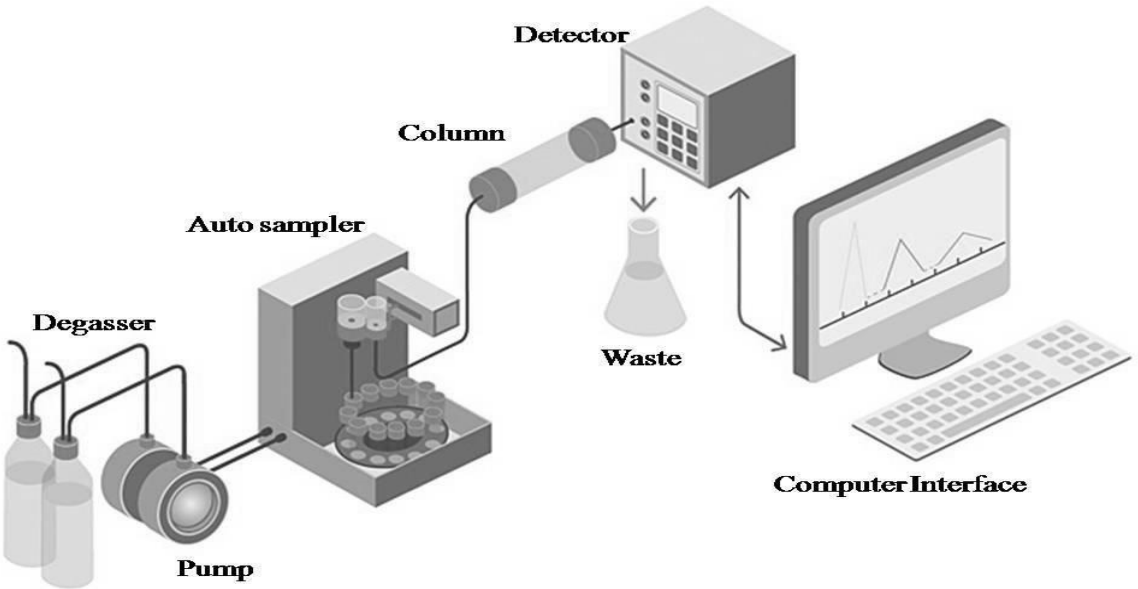


Figure 2: *Schematic diagram of instrumentation of HPLC [18].*

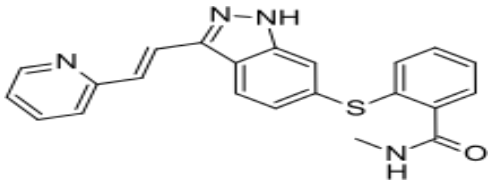
DRUG PROFILE

INTRODUCTION TO DRUG: [3]

Axitinib

Axitinib is an oral, potent, and selective inhibitor of vascular endothelial growth factor receptors (VEGFR) 1, 2, and 3. Axitinib is marketed with trade name Inayat®,

Structure:



Categories:

- Antineoplastic and Immunomodulating Agents
- Cytochrome P-450 CYP1A2 Substrates
- Cytochrome P-450 CYP2C19 Substrates
- Cytochrome P-450 CYP3A4 Substrates
- Enzyme Inhibitors

Iupac Name: [4, 5]

N-methyl-2-({3-[(E)-2-(pyridin-2-yl) Ethelyn]-2H-indazol-6-yl} sulfanyl) benzene-1-carboximidic acid

Molecular weight

386.47

Chemical formula

$C_{22}H_{18}N_4OS$

Mechanism of Action

Axitinib acts to mechanize in blocking the tyrosine kinase receptors (VEGFR-1, VEGFR-2, and VEGFR-3).

VEGFR- (vascular endothelial growth factor receptor).

Pharmacodynamics:

The Axitinib prevents the progression of cancer by inhibiting blocking tumor growth. And Angiogenesis

Indication: Used in kidney cell cancer and investigated for use/treatment in pancreatic and thyroid cancer.

MATERIALS AND METHODS [6, 7]

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	INERTSIL column, C18(150x4.6 ID) 5µm

Table 2: *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

Table 3: *Drug used.*

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

Mobile Phase [7, 8]
A mixture of 50 volumes of Triethylamine buffer pH 3.5 and 50 volumes of Acetonitrile was Prepared. 10 mins is the time duration for the mobile phase to sonicate and to remove the gases.

METHOD DEVELOPMENT AND VALIDATION

Introduction to Method Development [9,10]
New Drugs introduced to the market is raising high statistically every year. These drugs may be of either new entities or partial structurally modified 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. It happened because of the possible uncertainties in the continuation and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore, to develop newer analytical methods for such drugs.

Analytical methods were used to maintain good manufacturing practice (GMP) and good laboratory practice (GLP) proceeded according to International conference of Harmonization (ICH) guidelines

Method development is a continuous process that progresses in parallel with the evolution of the drug product. They should be suitable to support preclinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge. The methods are robust and uncomplicated, where they meet the appropriate regulatory guidelines. Scouting experiments are frequently performed during method development to establish the performance limits of the method, mainly for validation of experiments. These may include forced degradation studies, which are an integral part of the development of a stability-indicating method. API is subjected to degradation by acid, base, peroxide, heat, and light. This allows for a determination of the capability of the method to separate and quantify degradation products while providing in the main mechanisms of degradation. Once a stability-indicating method is placed in the formulated drug product can then be subjected to heat and light in order to evaluate the potential degradation of the API in the presence of formulation excipients. **Need for the**

Development of a New Method [11, 12]
Several reasons are available for the development of a new method of analysis.

- Existing methods may be too erroneous, artefact and/or contamination prone, or they may be unreliable (having poor accuracy or precision).
- Existing methods may be too expensive, time consuming, or energy intensive, or they may not be easily automated.
- Newer instrumentation and techniques may have evolved that provide opportunities for improved methods, including analyte identification or detection limits, greater accuracy or precision, or better return on investment.
- There may be a need for alternative method to confirm, for legal or scientific reasons, analytical data originally obtained by existing methods.

Method Development Using HPLC [13, 14] 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. Prior to method development there must be some technical information i.e. chromatography method selection according to the sample properties, the sample when analyzed with HPLC, the condition where all compounds elute in a reasonable time, optimization of HPLC method with regard to analysis time, resolution, selectivity and sensitivity.

Analyte Standard Characterization [15] All the information about the analyte is gathered regarding the structure, physical and chemical properties, toxicity, purity, hygroscopicity, solubility and stability. The availability of the 100% pure standard analyte is determined along with its storage and disposal information. If multiple components are to be analyzed in a sample matrix, the number of components to be analyzed is noted and the availability of the standard for each component is checked.

Literature Search and Prior Methodology [16] the available literature is searched for all types of information related to the analyte. Availability of information regarding the synthesis, physical and chemical properties, solubility or relevant analytical methods is determined. Books, periodicals, regulatory agency compendia, such as IP, USP/NF, BP etc. should be referred. Chemical Abstracts Service (CAS) automated/ computerized literature searches also should be used.

Information pertaining to prior analytical work on the analyte has to be determined within the company and compile the available data, results, reports, memos and publications.

Choosing a Suitable Method Using the available literature and previous methodology, the methods are adapted and modified. Sample preparation and instrument conditions are adopted to make use of the latest methods and instrumentation. Usually a compound with analytical method exists that is similar to the analyte of interest.
Optimization

Choice of method [9]
The most commonly used chromatographic methods are normal phase chromatography, reverse phase chromatography, reverse phase ion-pair chromatography and ion-exchange chromatography. In the selection of suitable chromatographic method for organic compounds, first reversed phase should be tried, if not successful, normal phase should be tried, then reverse phase ion-pair chromatography should be tried, ion-exchange chromatography at the end.

Choice of Mobile Phase
Selection of mobile phase is very important in the analysis of the drug in reversed phase chromatography. We can use acetonitrile frequently as it is suitable for the entire UV range, methanol and Isopropanol are not suitable below wavelength of 210nm, acetic acid is suitable above a wavelength of 240 nm, for the preparation of buffers, both $K_2HPO_4$and KH_2PO_4can be used in entire UV range, freshly distilled THF is suitable for HPLC above a wave length of 240nm, TEA is suitable above 240 nm, ammonium acetate can be used above 215 nm, EDTA can be used in entire UV range, sodium phosphate is suitable above 210 nm.

The required organic phase concentration for the mobile phase is estimated by

gradient elution method. For aqueous simple mixtures, the simple way to start is with gradient in reversed phase chromatography. Gradient can be started with 10% organic phase in the mobile phase and organic phase concentration (Methanol & Acetonitrile) can be increased up to 100% within 20-60 min. Separation can then be optimized by changing the initial mobile phase composition according to the chromatogram obtained from preliminary run. The initial mobile phase composition can be estimated on the basis of where the compounds of interest were eluted, at what mobile composition.

Changing the polarity of the mobile phase can alter the elution of drug molecules. The elution strength of a mobile phase is dependent on its polarity, the stronger the polarity, higher is the elution. Ionic samples (acidic or basic) can be separated, if they are presented in un-dissociated form. Dissociation of ionic samples may be suppressed by proper selection of ph.

The pH of the mobile phase has to be selected in such a way that the compounds are not ionized. If the retention time is too short, the decrease of the organic phase concentration in the mobile phase is of 5%. If the retention times are too long, an increase in 5% steps of the organic phase concentrations is needed.
When separating acid or bases, buffered mobile phase is required to maintain consistency in retention time and selectivity. Buffered salts reduce peak tailing for basic compounds by effectively masking silanol groups and also reduce potential ion- exchange interactions with a protonated silanol groups. As potassium is a stronger counter ion than sodium, it provides improved results compared to sodium (Na^+). Potassium phosphate is used for preparation of buffers of various pH. If band tailing is observed for basic amphoteric compounds few drops of diluted triethylamine or ammonium acetate is added, for acidic or amphoteric compounds, few drops of diluted triethylamine or ammonium acetate is tried. For neutral compounds, the aqueous eluent used in method development is water, for weak to medium acidic compounds, 100 mM H_3PO_4 buffer of pH 2.3, for weak to medium basic or acidic compounds in ionized form 100mM H_3PO_4 buffer of pH 4.0, 50 mM H_3PO_4buffer of pH 7.5 are used. Unknown sample should be analyzed first with water, then with an acidic and a neutral buffer. [7]

Choice of Column

Columns being the heart of HPLC for optimum separation, Stable, high performance column with good selectivity, efficiency is essential requirement for rugged and reproducible method. These characteristics are dependent on the columns manufacturer's production of good quality columns and packing materials.

Column length

- Longer columns are chosen for increased resolution.
- Shorter columns are chosen for shorter analysis time, lower back pressure, fast equilibration and less solvent consumption.

Column internal diameter

- Wider diameter columns are chosen for greater sample loading.
- Narrow columns are chosen for more sensitivity and reduced mobile phase consumption.

Particle shape

- Columns with spherical particle shapes are preferred when lower back pressure column stability and greater efficiency is required.
- Columns with irregular particle shapes are preferred when large surface area and high capacity is required.

Particle size

- Columns with small particle size of 3 - 4 µm are preferred for complex

mixtures with similar components. Combination of a short column (10- 50 mm) with small particle size is used for fast, high resolution separations.

- Columns with larger particle size of 5 – 10 µm are preferred for structurally different compounds.
- Columns with large particle of 15 – 20 µm are used for preparative separations.

Surface area

- Columns with high surface area packing are selected for more capacity, greater resolution and longer retention.
- Columns with low surface area packing are selected for quicker equilibration time.

Carbon load

- Columns with high carbon load are chosen for greater column capacities and resolution.
- Columns with low carbon load for faster analysis time.

a) Choice of Detector

Detectors are eyes of the liquid chromatography system and measure the compounds after their separation on the column. Selected detector should be capable of responding to change in concentrations of all the components in the sample with adequate sensitivity even to measure trace substances. The detectors must have certain characteristics i.e. high sensitivity, higher linear dynamic range, application to most of the solutes, does not contribute to band broadening, non-destructive, faster response.

Further Optimization [8]

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

For shorter analysis time

- Change to isocratic method. The suitable mobile phase composition is estimated from the gradient run.
- Use of shorter column, if proper resolution is obtained.

For better resolution

- Use of longer column.
- Use of stationary phase with smaller particles (3 – 4 µm).

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 lead to narrower and higher peaks as gradient elution, smaller particle, micro bore columns.

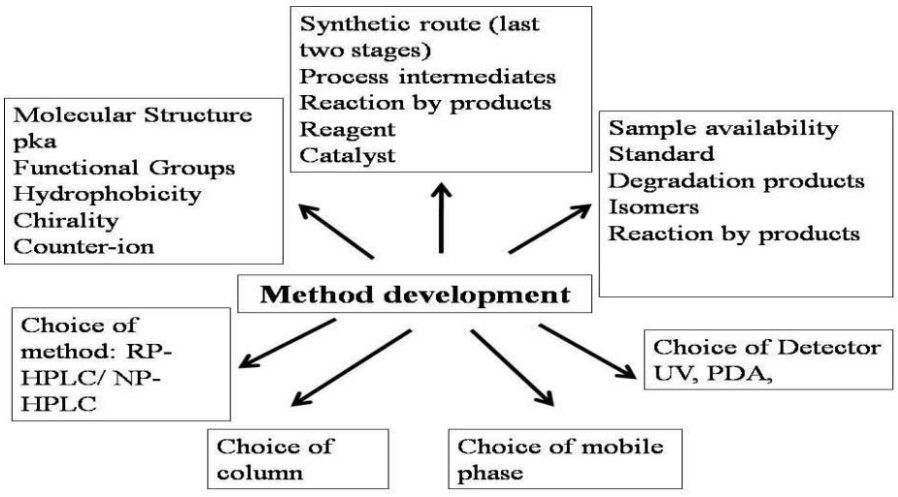


Figure 3: *Outline of the process involved in method development.*

Parameters Affecting Changes in Chromatograph [11]

The various parameters affecting the changes in chromatographic conditions are

1. Flow rate
2. Temperature
3. pH
4. Ion pair reagent
5. Column efficiency
6. Capacity factor
7. Resolution
8. Retention time
9. Peak asymmetry

Introduction to Method Validation

Validation is an integral part of quality assurance; it involves the systematic study of systems, facilities and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. Validation itself does not improve processes it should have been properly developed and are under control.

Method validation is defined as the process of proving that an analytical method is acceptable for its intended use. To ensure compliance with quality and safety standards, the United States, Europe, Japan, and other countries have published compendia, or pharmacopeias, that describe official test methods for many marketed drug products. For example, analytical methods found in United States Pharmacopeia (USP) are legally recognized analytical procedures under section 501 (b) of the Federal Food, Drug, and Cosmetic Act. A great deal of effort has been devoted to the harmonization of pharmaceutical regulatory requirements in the United States, Europe, and Japan. The recent FDA methods validation draft guidance documents as well as U.S. both refer to ICH guidelines.

The required validation parameters, also termed analytical performance characteristics or analytical figs of merit. Methods should be validated or revalidated. Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Method Validation (ICH Guidelines) [14]

1. Accuracy,
2. Precision,
   - Repeatability,
   - Intermediate precision.
3. Specificity / Selectivity,
4. Limit of Detection,
5. Limit of Quantitation,
6. Linearity,
7. Range,
8. Robustness,
9. System Suitability.

Table 4: *Acceptance Limits for System Suitability Test.*

Parameter	Limit
Capacity Factor	$k' > 2$
Injection precision	RSD < 1% for n ≥ 5
Resolution	$R_s > 2$
Tailing factor	$A_s \leq 2$
Theoretical plates	$N > 2000$

RESULTS AND DISCUSSION

Solubility Studies

These studies are carried out at 25 ^{0} C

AXITINIB: It is poorly soluble in water (1.0 mg/100 mL) and in aqueous solutions at low pH (0.1 mg/100 mL at pH 1.1 and 4.0; 0.2 mg/100 ml at pH 5.0). Solubility increases at higher pH values (in such where the preparations with 43 mg/100 mL at pH 7.5).

Determination of Working Wavelength (λmax)

In estimation of drug, wavelength is used.

Preparation of standard stock solution of

AXITINIB 10mg of **AXITINIB** was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Results- The wavelength of maximum absorption (Z_{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. The resulting spectra are shown in the fig. no. 8.1, 8.2 and 8.3 and the absorption curve shows characteristic absorption maxima at 254nm for AXITINIB

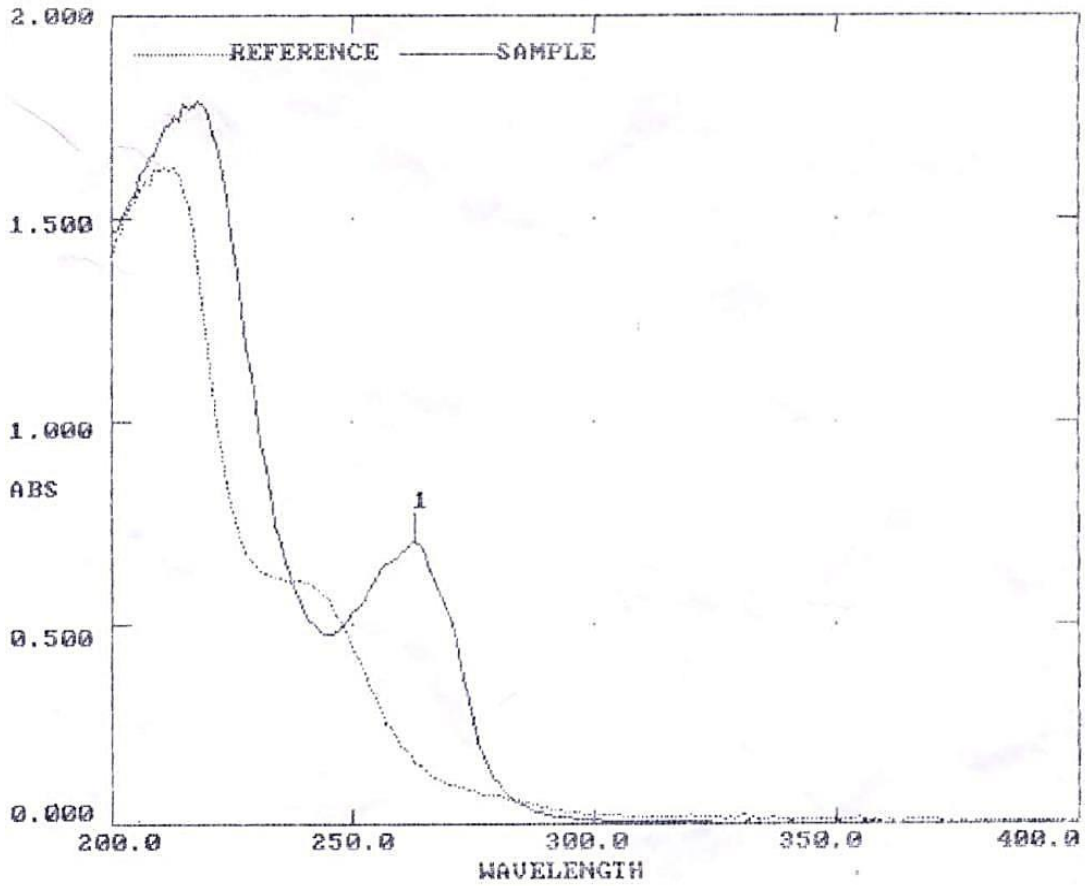


Figure 4: *UV-VIS spectrum of AXITINIB.*

Observation: Z_{max} was found to be 254 nm for AXITINIB shown in the figure

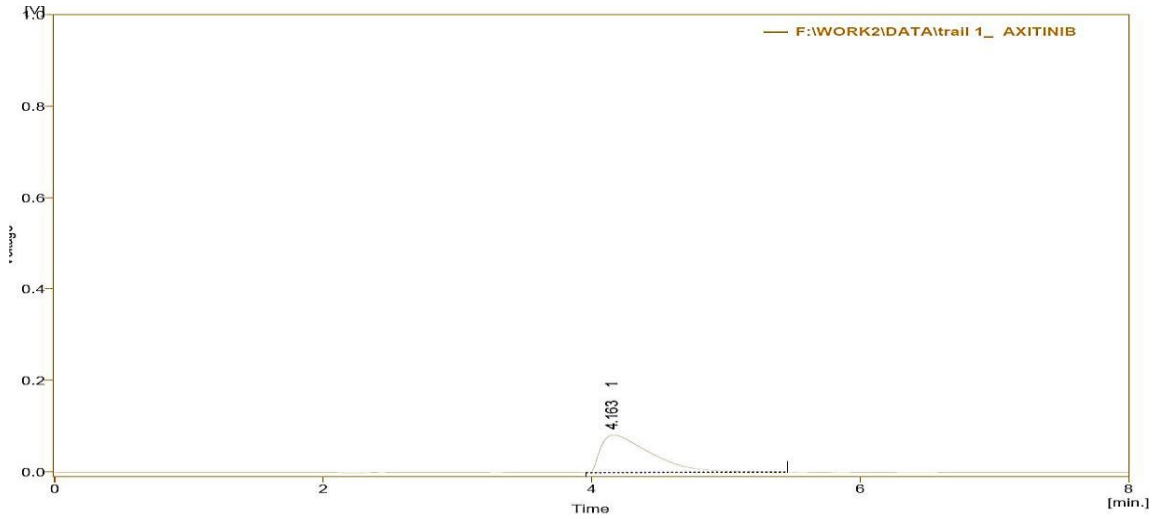
METHOD DEVELOPMENT OF AXITINIB. Trial - 1

Chromatographic conditions

Mobile phase : Mixed phosphate buffer: ACN (55:45) Column : Zodiac, C18 (250×4.6× 5µ)
wavelength : 254 nm
Flow rate : 1ml/min
Preparation of mixed 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.

Observation: The Asymmetry was not satisfactory for AXITINIB (Asymmetry more than 2 and greater than



Result Table (Uncal - F:\WORK2\DATA\trail 1_ AXITINIB

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.163	2154.104	82.131	100.00	100.0	0.40
	Total	2154.104	82.131	100.00	100.0	

Column Performance Table (From 50% - F:\WORK2\DATA\trail 1_ AXITINIB

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	4.163	0.403	4.000	0.00	590	11806	-

Trial- 2

Chromatographic conditions

Mobile phase : ACN: Water (40:60)

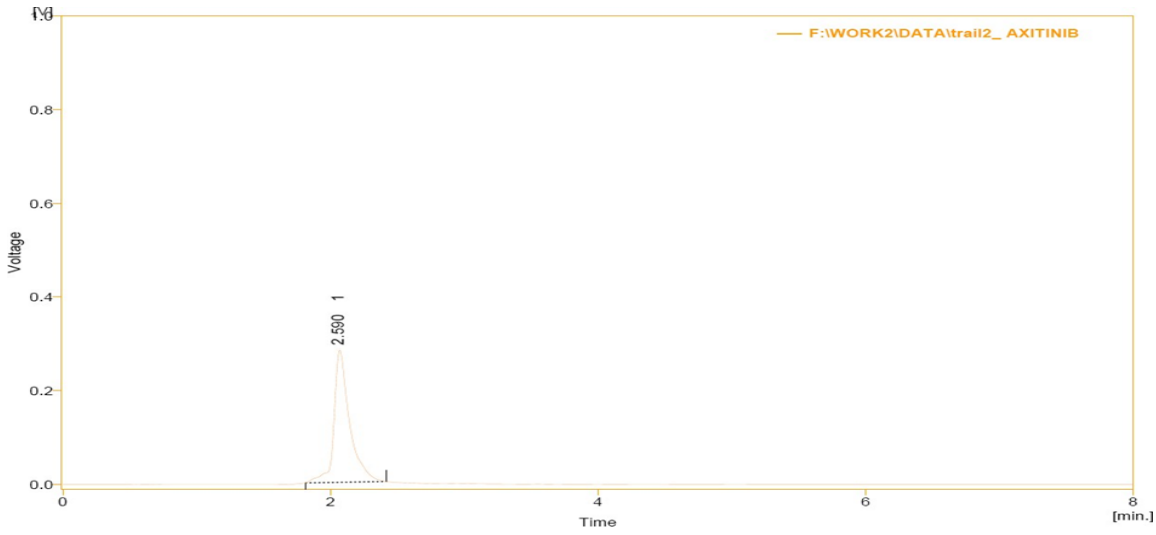
Column : Zodiac, C18 (250×4.6× 5µ)

Wavelength : 254nm

Flow rate : 1.2 ml/min

Preparation of mixed 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.



Result Table (Uncal - F:\WORK2\DATA\trail2_ AXITINIB

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.070	2297.753	282.117	100.00	100.0	0.11
	Total	2297.753	282.117	100.00	100.0	

Column Performance Table (From 50% - F:\WORK2\DATA\trail2_ AXITINIB

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.070	0.110	2.375	0.00	1962	39237	-

Observation: The Asymmetry for AXITINIB was more than 2.0. The efficiency of AXITINIB was less than 2000. The details are given in the table 8.3.2 and figure 8.3.2, Hence it was not taken for optimization.

Trial- 3:

Chromatographic conditions

Mobile phase : Mixed phosphate
Buffer : ACN +methanol (20+50+30)
Column : Zodiac, C18 (250×4.6× 5µ)
Wavelength : 254nm
Flow rate : 1ml/min

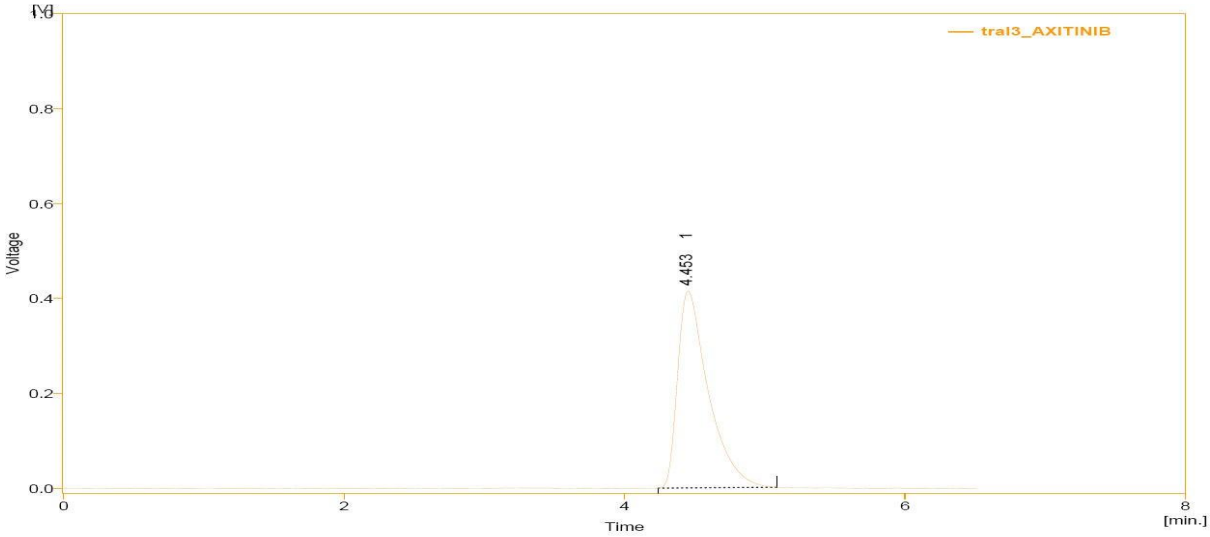
Preparation of mixed standard solution

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.

Chromatogram of AXITINIB by using mobile phase

Observation:

The Efficiency of AXITINIB was very less. Asymmetry was more



Result Table (Uncal - tral3_AXITINIB

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.453	6315.343	414.907	100.00	100.0	0.23
	Total	6315.343	414.907	100.00	100.0	

Column Performance Table (From 50% - tral3_AXITINIB

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	4.453	0.227	2.488	0.00	1138	21385	-

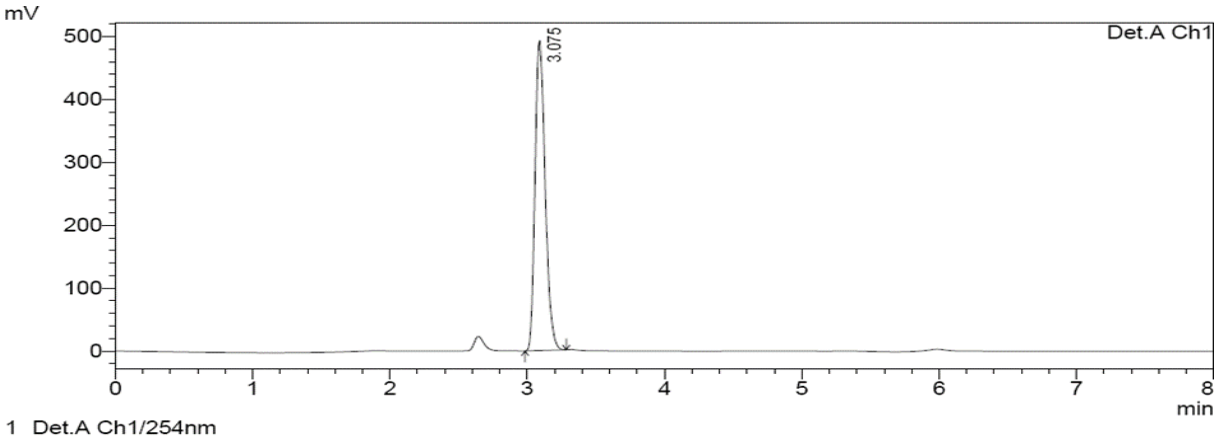
Trial - 4:

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

Preparation of mixed 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.



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		

Chromatogram of AXITINIB by using mobile phase.

Observation:

The peak efficiency was more than 2000. The peak tailing factor was less than 2.0.

The resolution between peaks was more than 1.5

Mobile phase	Triethylamine: Acetonitrile (50:50)
Column	Zodiac, C18 (250×4.6× 5µ)
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	254nm
Injection volume	20 µl
Run time	8 min
Retention time	About 3.075

Optimized chromatographic conditions

Assay

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	

Observation

The amount of AXITINIB present in the taken dosage form was found to be 90% and 110% respectively.

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
average	3.07867	2067229
Study	0.02863	15823.2
%RSD	0.92983	0.76543

Observation: Test results for AXITINIB was showing that the %RSD of Assay results are within limits

Result of Robustness study

Parameter	AXITINIB	
	Retention time(min)	Area
Flow		
1.0ml/min	3.671	2589974
1.4ml/min	2.696	1891623
Wavelength		
252nm	3.081	2113775
256nm	3.086	2154238

Observation: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Results for Ruggedness

AXITINIB	%Assay
Analyst 01	99.07325
Analyst 02	99.79389

Observation: From the observation the %RSD between two analysts Assay values were not greater than 2.0%, hence the method is rugged.

SUMMARY

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 drug estimated by the proposed methods was in good agreement with the label claim.

The proposed method was validated. The accuracy of the methods was assessed by recovery studies at three different levels. Showing %RSD less than. All statistical data proves validity of 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 estimation of AXITINIB was found to be simple, precise, accurate and high resolution and lower retention time makes this method more acceptable and effective and it can be applied for routine analysis in research institutions for quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-

equivalence studies and in clinical pharmacokinetic studies in near future.

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