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METHOD DEVELOPMENT AND ITS VALIDATION FOR ANTINEOPLASTIC AGENTS (RUXOLITINIB) BY RP-HPLC

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ABSTRACT

A sensitive& selective RP-HPLC method has been developed & validated for the analysis of Ruxolitinib. Encourage the proposed RP-HPLC technique has astounding affectability, accuracy and reproducibility. The outcome demonstrates the created technique is amazingly, one more appropriate strategy for test, immaculateness and solidness which can help in the examination of Ruxolitinib in various definitions.

Keywords: RP-HPLC**,** Ruxolitinib, Method development, Validation parameters

INTRODUCTION

High Performance Liquid Chromatography: High Performance Liquid Chromatography (HPLC) is a type of fluid chromatography to isolate exacerbates that are broken down in arrangement. HPLC instrument comprise of a repository of portable stage, a pump, an injector, a partition segment, and a finder**.**

High execution fluid chromatography (HPLC) is fundamentally an exceptionally enhanced type of section fluid chromatography. Rather than a dissolvable being permitted to dribble through a section under gravity, it is constrained through under high weights of up to 400 environments. That makes it considerably quicker. Every single chromatographic partition, including HPLC work under a similar fundamental standard; detachment of an example into its constituent parts due to the distinction in the relative affinities of various particles for the portable stage and the stationary stage utilized in the division[1].

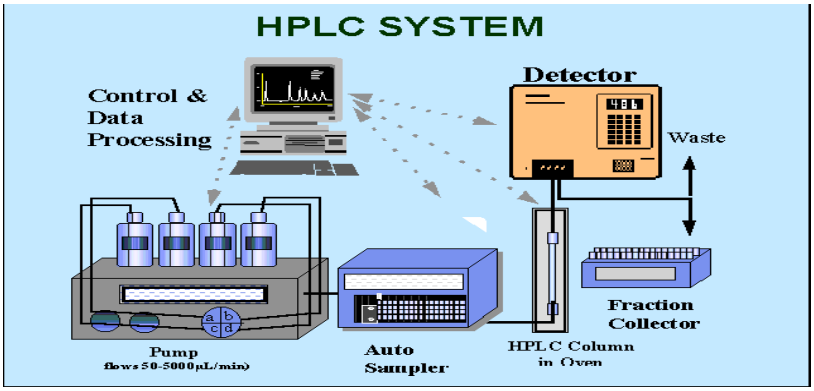


Fig 1: Schematic Chart of HPLC

There are following variations of HPLC, contingent on the stage framework (stationary) all the while: Normal Phase HPLC: This technique isolates analytes based on extremity. NP-HPLC utilizes polar stationary stage and non-polar portable stage. Hence, the stationary stage is generally silica and run of the mill versatile stages are hexane, Methylene chloride, chloroform, diethyl ether, and blends of these. Polar examples are therefore held on the polar surface of the segment pressing longer than less polar materials[2]. Reverse Phase HPLC: The stationary stage is nonpolar (hydrophobic) in nature, while the portable stage is a polar fluid, for example, blends of water and methanol or acetonitrile. It deals with the rule of hydrophobic co-operations henceforth the more nonpolar the material is, the more it will be held.

METHOD DEVELOPMENT

A technique is an arrangement of exploratory conditions intended to make a decent investigation of a specific example.

Method development includes numerous stages and can take a very long time to finish, contingent upon the many-sided quality and objectives of the technique [3].

METHOD VALIDATION

Approval of an investigative method is the procedure by which it is set up, by research facility ponders, that the execution attributes of the strategy meet the prerequisites for its expected utilize. The strategies approval process for expository methodology starts with the arranged and precise gathering by the candidate of the approval information to help systematic techniques. Every single explanatory technique that are proposed to be utilized for breaking down any clinical examples should be approved. The approval of diagnostic strategies is done according to ICH rules [4].

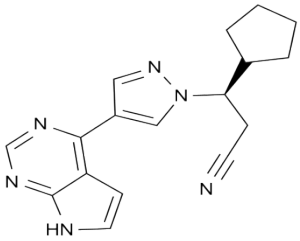
METHOD VALIDATION PARAMETERS [5-10].

The accompanying are normal systematic execution qualitie which might be tried amid techniques approval: Accuracy, Precision, Repeatability, Intermediate precision, Linearity, Detection limit, Quantization limit, Specificity, Range, Robustness, System suitability determination, Precision, measures of analyte. Exactness of an expository technique is the level of assertion among singular test outcomes got when the strategy is connected to different examining of a homogenous example [5].

DRUG PROFILE

Name [11]: RUXOLITINIB

Chemical Structure:



Description: Ruxolitinib is a janus-associated kinase inhibitor indicated to treat bone marrow cancer, specifically intermediate or high-risk myelofibrosis. FDA approved on November 16, 2011.

Categories: Antineoplastic Agents, Immunosuppressive Agents, Myelosuppressive Agents.

Chemical Formula: $C_{17}H_{18}N_{6,}$

Molecular Weight: Average: 306.365 g/mol

Indication[12]: Treatment of intermediate or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera (post-PV) myelofibrosis and post-essential thrombocythemia (post-ET) myelofibrosis. Myeolofibrosis is the proliferation of abnormal bone marrow stem cells which cause fibrosis (the excessive formation of connective tissue)[13].

METHOD DEVELOPMENT

LIST OF INSTRUMENT USED

S. No.	Instruments/Equipments/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	T60-LAB INDIA UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5❍m, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

CHEMICALS / REAGENTS USED

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen	96%	A.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
6.	Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
7.	Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
8.	Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

SOLUBILITY STUDY

DMSO- Soluble, DMF- Soluble, Water- Soluble, Methanol- Freely Soluble, Dichloro Methane- Slightly Soluble, Acetonitrile- Soluble

METHOD DEVELOPMENT AND ITS VALIDATION FOR RUXOLITINIB BY RP-HPLC

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Ruxolitinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Ruxolitinib. The scanned UV spectrum is attached in the following page.

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Ruxolitinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

METHOD VALIDATION

1.Accuracy

To decide the exactness of the proposed strategy, recuperation thinks about were done by including diverse sums (80%, 100%, and 120%) of unadulterated medication of RUXOLITINIB were taken and added to the pre-broke down plan of fixation 10µg/ml. From that rate recuperation esteems were computed.

2.Precision

2.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Ruxolitinib (API). The percent relative standard deviation was calculated for Ruxolitinib are presented in the table.

Table 1: Readings of Repeatability

HPLC Injection Replicates of Ruxolitinib	**Retention Time (Minutes)**	**Peak Area (AUC)**
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		**197121.5**
Standard Deviation		**1515.213**
% RSD		**0.768667**

2.2. Intermediate Precision

2.2.1. Intra-assay & inter-assay

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Ruxolitinib revealed that the proposed method is precise.

Table 2: Results of Intra-Assay & Inter-Assay

Conc. Of Ruxolitinib(API) (µg/ml)	**Observed Conc. Of Ruxolitinib (µg/ml) by the proposed method**			
	Intra-Day		**Inter-Day**	
	Mean (n=6)	**% RSD**	**Mean (n=6)**	**% RSD**
8	7.46	0.62	8.05	0.96
10	10.87	0.85	9.43	0.71
12	11.81	0.92	12.04	0.65

3. Linearity & Range

The calibration curve showed good linearity in the range of 6 – 14 µg/ml, for Ruxolitinib (API) with correlation coefficient (r^2) of 0.999 (Fig-6.16). A typical calibration curve has the regression equation of y = 19423x + 5444 for Ruxolitinib.

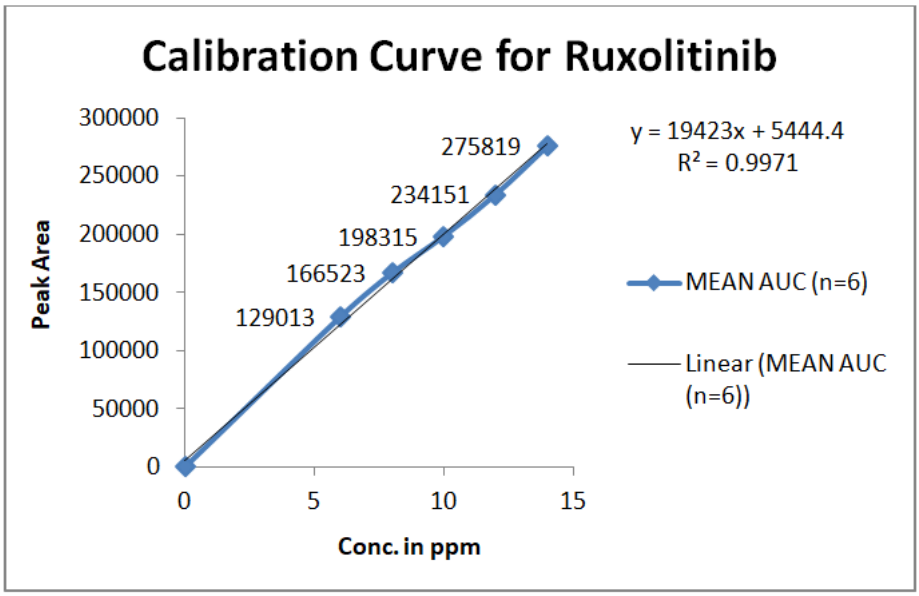


Fig 2: Calibration Curve of Ruxolitinib (API).

4. Method Robustness

Influence of small changes in chromatographic conditions such as change in flow rate (⌖ 0.1ml/min), Wavelength of detection (⌖2nm) & organic phase in mobile phase (⌖5%) studied to determine the robustness of the method are also in favour of (Table-6.25, % RSD < 2%) the developed RP-HPLC method for the analysis of Ruxolitinib (API).

Table 3: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.68
Flow (0.9 ml/min)	0.39
More Organic	0.54
Less Organic	0.63
Wavelength of Detection (237 nm)	0.91
Wavelength of detection (233 nm)	0.93

5. LOD & LOQ

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

6. System Suitability Parameter

Framework appropriateness testing is an essential piece of numerous scientific techniques. The tests depend on the idea that the gear, hardware, explanatory activities and tests to be broke down establish a vital framework that can be assessed all things considered. Following framework appropriateness test parameters were built up. The information is appeared in Table.

Table 4: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs ✇ 2	8.47
2	Asymmetry	T ✪ 2	Ruxolitinib=0.23
3	Theoretical plate	N ✇ 2000	Ruxolitinib=2987
4	Tailing Factor	T<2	Ruxolitinib=1.17

7. Estimation of Ruxolitinib in Pharmaceutical Dosage Form

Label claim: Each tablet contains: 20 mg: Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after to decide the normal weight. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 m) and sonicated to degas. The arrangement arranged was infused in five reproduces into the HPLC framework and the perceptions were recorded. A copy infusion of the standard arrangement was additionally infused into the HPLC framework and the peak regions were recorded.

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where,
AT = Peak Area of medication acquired with test arrangement, AS = Peak Area of medication acquired with standard arrangement, WS = Weight of working standard taken in mg
WT = Weight of test taken in mg, DS = Dilution of Standard arrangement, DT = Dilution of test arrangement, P = Percentage virtue of working standard.

Table 5: Recovery Data for estimation Ruxolitinib in Jakavi

Brand Name of Ruxolitinib	Labelled amount of Drug (mg)	Mean (⌖ SD) amount (mg) found by the proposed method (n=6)	Assay % (⌖ SD)
Jakavi (20mg) (Neelkanth Pharmaceuticals Private Limited)	20mg	19.58 (⌖0.627)	99.79 (⌖0.277)

STABILITY STUDIES

Following convention was entirely clung to for constrained corruption of Ruxolitinib Active Pharmaceutical Ingredient (API). The API (Ruxolitinib) was subjected to pressure conditions in different approaches to watch the rate and degree of debasement that is probably going to happen over the span of capacity as well as after organization to body. This is one kind of quickened strength contemplates that causes us deciding the destiny of the medication that is probably going to occur after prolonged stretch of time stockpiling, inside a brief timeframe as contrast with the continuous or long haul soundness testing. The different corruption pathways examined are corrosive (Acid) hydrolysis, essential (Base) hydrolysis, warm debasement (Thermal), photolytic corruption and oxidative debasement. The results of the stress studies indicated the specificity of the method that has been developed. Ruxolitinib was stable in thermal and photolytic stress conditions. The result of forced degradation studies are given in the following table 6.

Table 6: Results of forced degradation studies of Ruxolitinib API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	81.36	18.64	100.0
Basic Hydrolysis (0.I M NaOH)	24Hrs.	83.37	16.63	100.0
Thermal Degradation (50 ^{0}C)	24Hrs.	98.92	1.08	100.0
UV (254nm)	24Hrs.	96.33	3.67	100.0
3 % Hydrogen peroxide	24Hrs.	89.41	10.59	100.0

RESULTS AND DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Ruxolitinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS RP C_{18}, 5❍m, 15mmx4.6mm i.d. Column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). The drug was found to be Soluble in DMSO, ethanol, DMF, water, methanol and acetonitrile and Slightly soluble in dichloro methane. Utilizing these solvents with suitable arrangement more current techniques can be created and approved. Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Ruxolitinib it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Ruxolitinib in various details.

CONCLUSION

A sensitive& selective RP-HPLC method has been developed & validated for the analysis of Ruxolitinib. Encourage the proposed RP-HPLC technique has astounding affectability, accuracy and reproducibility. The outcome demonstrates the created technique is amazingly, one more appropriate strategy for test, immaculateness and solidness which can help in the examination of Ruxolitinib in various definitions.

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