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Research article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF FEDRATINIB IN PHARMACEUTICAL DOSAGE FORM BY HPLC

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Abstract

Accurate, and sensitive HPLC technique has been created to estimate fedratinib in medication dosages. Form utilising a Shimadzu C18 column (250 mm× 4.6 mm, 5 µm) with a mobile phase consisting of HPLC-grade acetonitrile and Phosphate Buffer at pH 5.0. A 0.45µm membrane filter was used tofilter the mobile phase at a flow rate of 1.0 mL/min after it had been sonicated for 10 minutes. The fedratinib retention period was determined to be 2.90minutes when the detection was done at 290 nm. Regression equation y = 10527x - 18715 showed linearity in the concentration range of 25-75 µg/mL(coefficient of determination R2=0.9987). Method validation followed ICH guidelines.

Key words: Fedratinib, RP-HPLC Method, Validation.

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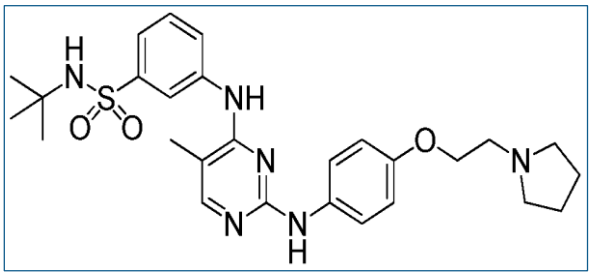
Introduction [1,2]

N-tert-butyl-3-[[5-methyl-2-[4-[2-(1-pyrrolidinyl) ethoxy] anilino] is the chemical formula for fedratinib. Semi-selective inhibitor of Janus kinase 2 (JAK-2) is 4-pyrimidinyl] amino] benzenesulfonamide used to treat myelofibrosis [3][4].

Figure 1: Structure of Fedratinib



According to the literature, fedratinib has been developed. This effort aimed to design and validate an HPLC technique [3–14] that was straightforward, specific, sensitive, accurate, and precise for the estimation of fedratinib in bulk and in its capsule dosage form**.**

Materials and Methods

Materials

Glyra Healthcare provided a complimentary sample of fedratinib working standard. The fedratinib capsule formulation produced by Glyra Healthcare was bought at the neighbourhood market.

Chemicals and reagents

Fedratinib was purchased from Sunrise Remedies. Finar Chemical Ltd. in Ahmedabad provided the analytical grade ortho-phosphoric acid and HPLC grade methanol. “Acetonitrile of HPLC grade was acquired from” Finar Limited in Gujarat. Ranbaxy Chemicals, located in New Delhi, provided AR grade potassium di-hydrogen orthophosphate (KH2 PO4) for purchase.

Instrumentation

For spectrum measurements, an FTIR Bruker, Alpha-II, and a Shimadzu HPLC LC-20 were utilised. To weigh the reagents, an analytical balance with extreme precision was employed. The medication was solubilised using ultrasonication.

Chromatographic conditions

An LC-20 AT importance solvent delivery module, 20µL fixed loop with manual injector and HPLC system with a UV detector. At room temperature, the estimation was performed on a Shimadzu C18 column (250 mm × 4.6 mm, 5 µm)”. For the preparation of mobile phase 800 mL of HPLC-grade acetonitrile and 200 mL of pH 5.0 HPLC-grade buffer was made.” “After a 10-minute sonication, with the help of 0.45µm membrane filter, the mobile phase was filtered. Eluents were seen at 290 nm, and the flow rate of mobile phase was kept continuous at 1 mL per min. A 20µL fixed loop was used to inject the samples. For a duration of six minutes, all calculations were carried out at room temperature.

Table 1: The optimized chromatographic conditions

Parameters	Conditions
Stationary phase (column)	Shimadzu (250 mm × 4.6 mm, 5 µm)
Mobile phase ratio	Phosphate Buffer, pH 5.0: Acetonitrile (20:80 v/v)
Detection Wavelength	290 nm
Flow Rate	1.0 ml/min
Injection Volume	20 µL
Column temperature	Ambient temperature
Run Time (min)	6 min
Retention time (min)	2.920

Method development

- **Selection and preparation of mobile phase**
  Different mobile phases with varying percentages of methanol, acetonitrile, water, and phosphate buffer (pH 3) were tested at various flow rates. A well-symmetrical peak was discovered when the “mobile phase was composed” of 20:80 (v/v) acetonitrile to pH 5.0 phosphate buffer. “The mobile phase was made by combining 800 millilitres of HPLC-grade acetonitrile with 200 millilitres of phosphate buffer (pH 5.0). After 10 minutes of sonication, and filter the mobile phase by using a 0.45µm membrane filter.
- **Preparation of stock solution**
  Fedratinib's “standard stock” solution was made by weighing “50 mg of the medication and transferring it to a 100 mL volumetric flask”. Methanol was then added to reach the volume mark, yielding a concentration of 500 µg/ml.
- **Preparation of calibration curve**
  In specific trials, aliquots of 1 ml of “standard stock solutions were transferred” to a 10 mL volumetric flask and diluted with methanol up to the appropriate level. “Plotting the peak area on the y-axis against” the corresponding drug “concentration on the x-axis” allowed for the construction of the calibration curve.
- **System suitability tests**
  To conduct the system appropriateness testing, information was gathered from six duplicate injections of a typical medication solution.
- **Analysis of Pharmaceutical Formulation**
  After precisely weighing tablet capsule powder equal to 50 mg of fedratinib, it was placed into a 100 ml volumetric flask, and then 60 ml of “mobile phase was added, and the mixture was shaken for 15” minutes before being diluted with mobile phase. (500µg/mL stock solution). Mobile phase was used to further dilute this solution until it reached a concentration of 50µg/mL. The solution

was fed into the HPLC apparatus in a volume of 50µL.

Method validation [15]

By assessing specificity, accuracy, precision, linearity, detection limit, quantification limit, robustness, and ruggedness, the developed method was validated. A fewer than 2% coefficient of variation and relative errors were deemed acceptable, with the exception of the quantification and detection limits.[22]

- **Specificity**
  By recording the chromatogram of the mobile phase, standard solution, and sample solution at 50µg/mL concentrations to identify any excipient interference in the sample, the specificity of the procedure was ascertained.
- **Accuracy**
  By calculating recovery, the accuracy of the approach was found at the 80%, 100%, and 120% levels. Each solution was injected three times, with a known quantity of standard solution added, and the percentage recovery was computed. A dose of the medication solution, labelled A, B, and C, was taken in three separate flasks. Dilution up to 10ml with 80%, 100%, and 120% of the standard solution spiked in it. At 290 nm, the area of every solution peak was calculated. Fedratinib dosages at each level were determined, and recoveries as a percentage were computed.
  Acceptance standards: % Individual recovery for each level should range from 98% to 102%.
- **Precision**
  The peak area, which was achieved by actually determining “six replicates of a given amount” of the medication “(50µg/mL)”, was used for the determination of the method's precision. The assay's precision was assessed by examining the variations in the peak regions of the drug solutions on three distinct days, both within and between days. “Relative standard deviation (RSD) was” used to compute “the intra- and inter-day” variation in the drug solution's peak area. Fedratinib's percentage RSD was discovered to be 0.052.
- **Linearity**
  By analysing the combined “standard solution” in the “range of 25-75 µg”/ml for Fedratinib, the “linearity” of the drug “was evaluated”. Pipette-out 5,7.5,10,12.5, and 15 ml solutions from the Fedratinib “stock solution” (500 µg/ml) into a “10-ml volumetric flask”. Then, add “mobile phase to the” flask to get 25,37.5,50,62.5, and 75 µg/ml of Fedratinib.
  “The graph” of the acquired “peak area” vs the corresponding “concentration was plotted” against in terms of slope, intercept, and correlation coefficient value.
  It was discovered that the calibration curve Fedratinib's correlation coefficient was 0.9987.
  Fedratinib's regression line equation looks like this:
  Fedratinib's y = 10527x – 18715
- **Detection limit and quantification limit**[28]
  The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formula: LOD is equal to 3.3SD per slope and LOQ is equal to 10SD per slope
- **Robustness**
  Robustness of the proposed method for Fedratinib “was carried out by the slight variation in flow rate”. The % recovery and RSD were noted for Fedratinib**.**

Results

Method development

- **Chromatographic determination**
  A variety of “HPLC chromatographic” methods were, examined in order to maximise Fedratinib determination. Fedratinib retention period. Table 1 displays the operation of the mobile phase, other optimal chromatographic settings, and the “stationary phase” (Shimadzu “C18 reversed-phase column”). Table 2 displays the outcomes of system appropriateness tests. The R.S.D. of the conventional Fedratinib area in system appropriateness was determined to be 0.052%. It was discovered that the retention duration, theoretical plate number, and tailing factor were, respectively, 2.920 min, 10045, and 1.008. The results showed that the system was appropriate for analysing these medications.
- **Calibration curve and analysis of pharmaceutical formulation**
  Fedratinib's slope, intercept, and coefficient of determination (R2) are all 0.998. Fedratinib had a retention period of 2.920 minutes. Figures 2 and 3 display the calibration curve and representative chromatogram of fedratinib at 290 nm, respectively. Fedratinib percentage assay was found to be 99.6% w/w in tablets that were marketed. It thereby surpasses the assay limit.

Table 2 System Suitability Parameters

Parameters	Limits	Fedratinib
Retention Time	-	2.920
Theoretical Plates	More than 2000	10045
Asymmetry	Not more than 2	1.07

Method validation

- **Accuracy, precision and linearity**
  The percentage recovery served as a gauge for the method's accuracy. Table 3 presents the findings. Tables 4 and 5 present the intra-day and “inter-day precision data” obtained using “the RP-HPLC method for” Fedratinib, respectively. Fedratinib's coefficient of determination (R2) in terms of linearity was 0.9987, as indicated by Figure 2 and Table 6.

Figure 2: Calibration curve of Fedratinib

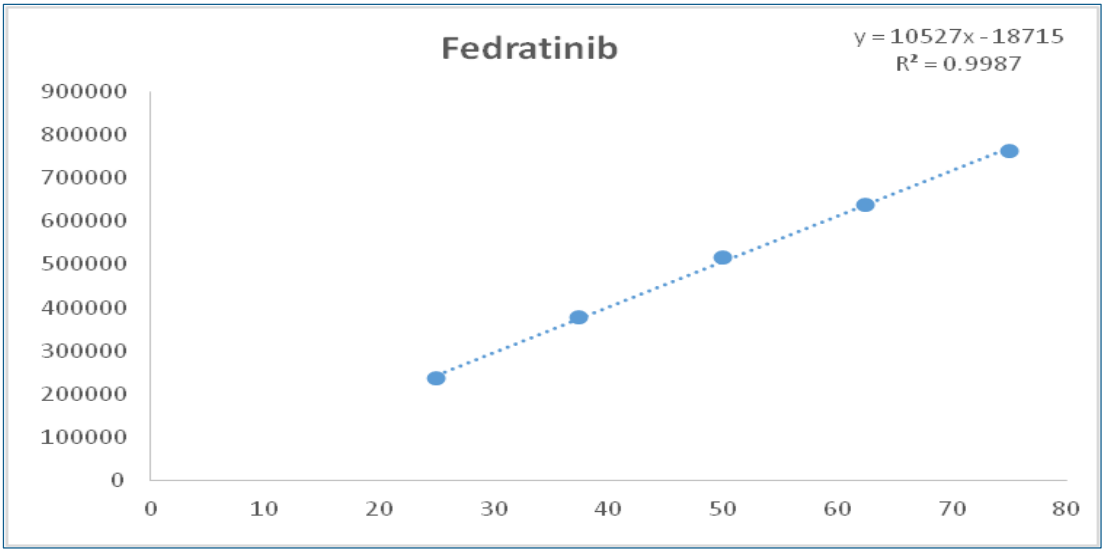


Figure 3: Representative chromatogram of Fedratinib

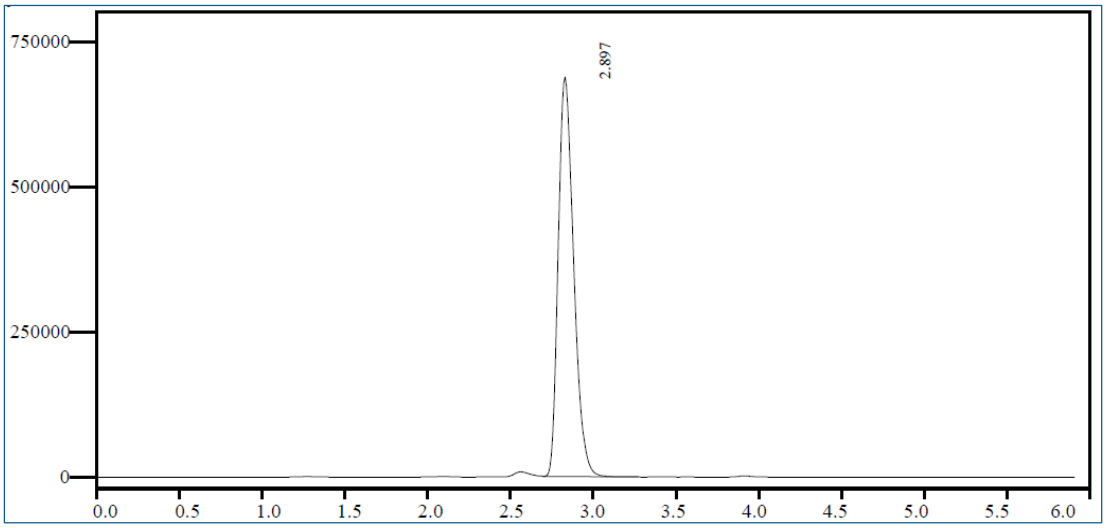


Table 3: Accuracy study of Fedratinib

Sr. No.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% RSD
1	80 %	25	20	20.000	100.000	0.506
2		25	20	20.010	100.049	
3		25	20	20.181	100.903	
4	100 %	25	25	24.996	99.983	0.390
5		25	25	24.809	99.234	
6		25	25	24.947	99.786	
7	120 %	25	30	30.000	100.001	0.156
8		25	30	30.071	100.237	
9		25	30	30.089	100.297	

Table 4: Intra-day precision of Fedratinib (n=3)

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=3)	% R.S.D
1	25	236181±912.17	0.386
2	50	518899±1514.62	0.292
3	75	776536±8315.47	1.071

Table 5: Interday data for Fedratinib

Sr. No.	Conc. (µg/ml)	Mean ± S.D (n=3)	% R.S. D
1	25	238869±2051.06	0.858
2	50	508005±8987.14	1.769
3	75	787981±9784.75	1.291

Table 6: linearity of Fedratinib (n=3)

Sr. No	Concentration (µg/ml)	Area
1	25	236589
2	37.5	379756
3	50	517985
4	62.5	638952
5	75	764941
Regression line		y = 10527x – 18715
Correlation co-efficient		0.9987

- **LOD and LOQ**
  In the present study the LOD was 2.67 µg/ml and LOQ was 8.09 µg/ml of Fedratinib.
- **Robustness**
  In the present study, robustness of the method was performed by deliberate variations of the analytical parameter such as flow rate 1±0.2mL per min. The results are given in the Table 7.

Discussion

A HPLC method for estimating fedratinib in capsule dose form was devised and validated in the current study. "The mobile phase, Phosphate Buffer, pH 5.0: Acetonitrile (20:80 v/v), was used at a flow rate of 1.0 mL/min to create an HPLC method for the estimation of fedratinib in capsule dosage form." The estimate was, performed using, a column, Shimadzu C18, (250mm, 4.5mm, 5µm). 290 nm was used as the detecting wavelength.

Then, using predetermined parameters, these "samples were examined using the HPLC method,"and the findings were, examined and verified. 99.6%w/w was found to be the percentage assay of the marketed capsule. Here, the regression line equation was determined as per y = 10527x – 18715. The results showed that the approach was "linear in the concentration range of 25–275g/mL with R 2 value 0.998." The results for precision and repeatability indicated that the tailing factor was less than 2, the LOD was 2.67 µg/ml, the LOQ was 8.09 µg/ml, and the percent RSD was less than 2%.

Conclusion

Here, the confirmed HPLC techniques used turned out to be straightforward, precise, accurate, and specific. The approaches that work well for analysing fedratinib in bulk drug and in capsule formulation without interference from excipients was demonstrated by statistical analysis. Therefore, Fedratinib in API and its capsule dosage forms can be routinely analysed using the suggested procedures.

Table 7: Robustness of Fedratinib

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase (-2)	Area at Mobile phase (+2)	Area at pH (- 0.2)	Area at pH (+0.2)
1	380564	379684	375948	375145	389583	385489
2	375146	385413	385684	384578	390585	374589
3	369478	386126	378547	378859	388840	385489
% R.S. D	1.478	0.920	1.323	1.252	0.224	1.648

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