ISSN 2319-2119

RESEARCH ARTICLE

GUDISA.KISHORE, The *Experiment, October.2012 Vol.3 (1), 174 -181*

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

A VALIDATED RP-HPLC METHOD FOR ESTIMATION OF REGORAFENIB IN BULK AND TABLET DOSAGE FORM

ABSTRACT

A simple, selective, linear and accurate isocratic RP-HPLC method has been developed for the estimation of Regorafenib in and tablet dosage forms. The chromatographic separation was achieved on C18, 250×4.60mm 5micron particle size column. The mobile phase consists of a mixture of acetonitrile and Methanol (pH 5.2) in the ratio of 45:55% v/v with UV-detection at 261 nm. The flow rate was kept at 1mL/min. The method shows a linear response for concentrations in the range of 5-25µg/mL. The developed method was statistically validated for Accuracy, linearity, precision, robustness, LOD and LOQ.

KEY WORDS: Regorafenib C18 column, Method development and validation, RP-HPLC,261 nm

INTRODUCTION

Regorafenib is an oral multi-kinase inhibitor developed by Bayer which targets angiogenic, stromal and oncogenic receptor tyrosine kinase . Regorafenib shows anti-angiogenic activity due to its dual targeted VEGFR2-TIE2 tyrosine kinase inhibition. It is currently being studied as a potential treatment option in multiple tumor types.[1] Regorafenib demonstrated to increase the overall survival of patients with metastatic colorectal cancer[2-3] Stivarga is being approved with a Boxed Warning alerting patients and health care professionals that severe and fatal liver toxicity occurred in patients treated with Stivarga during clinical studies. The most common side effects reported in patients treated with Stivarga include weakness or fatigue, loss of appetite, hand-foot syndrome (also called palmar-plantar erythrodysesthesia), diarrhea, mouth sores (mucositis), weight loss, infection, high blood pressure, and changes in voice volume or quality (dysphonia).[4]

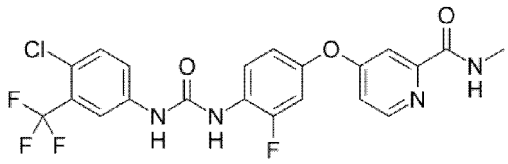

Fig.1 Structure of Regorafenib

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Materials

Working standard of Regorefenib was obtained from well reputed research laboratories. Acetonitrile, Methanol,OPA was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

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Determination of wavelength of maximum absorbance

The standard solutions of Regorefenib were scanned in the range of 200 -400 nm against mobile phase as a blank. Regorefenib showed maximum absorbance at 261 nm. So the wavelength selected for the determination of Regorefenib was 261 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisted of a Methanol: Acetonitrile: 55:45 (v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 261 nm with 10 min runtime.

Standard and sample solutions

A 10 mg amount of Regorefenib reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations including standard concentration of 40 ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

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S.NO	Parameter	Condition
1	Mobile phase	Methanol: Acetonitrile: 55:45 (v/v)
2	Pump mode	Isocratic
3	pH	5.2
4	Diluents	Mobile phase
5	Column	Chromosil C18 column (250 X 4.6 mm, 5µ)
6	Column Temp	Ambient
7	Wavelength	261 nm
8	Injection Volume	20 µl
9	Flow rate	1 ml/min
10	Run time	10 minutes
11	Retention Time	6.49 minutes

Table.1 System suitability parameters

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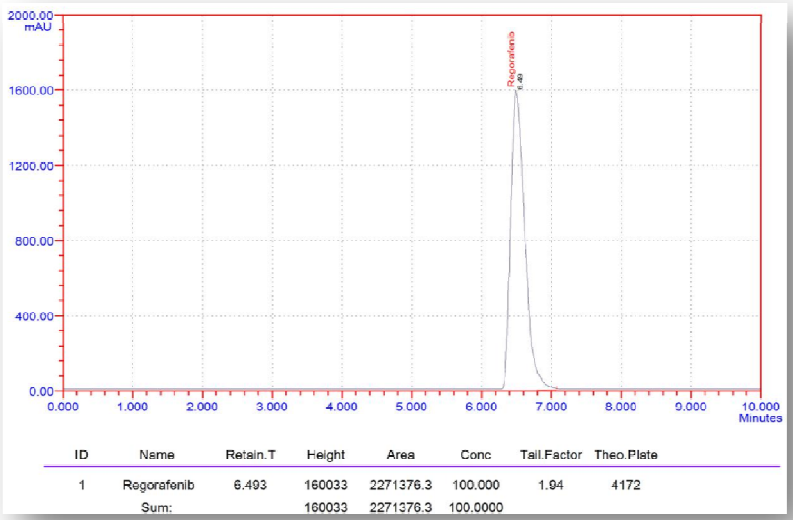

Figure.2 Standard chromatogram

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 30,60,90,120,150 and 180 ppm for Regorefenib. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -15680.8 + 56589.36x (r= 0.999). Linearity values can shown in Table: 2

LEVEL	CONCENTRATION OF REGOREFENIB IN PPM	PEAK AREA
Level 1	0	0
Level 2	10	501278
Level 3	20	1127639
Level 4	30	1728497
Level 5	40	2271376
Level 6	50	3395213
Level 7	60	3954678
	70	3954678
Range 10-70 ppm	SLOPE INTERCEPT CORREALATION COEFFICIENT	56589.36 -15680.8 0.99912

Table.2 Linearity Range

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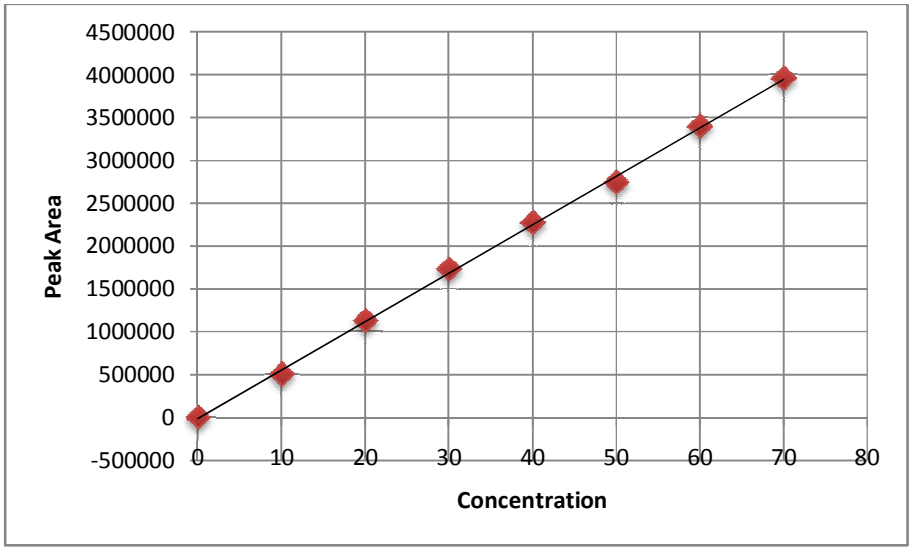

Graph.1 Calibration curve

Precision

To study precision, six replicate standard solutions of Regorefenib (120 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Precision Results for Regorefenib:

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTER DAY RSD (Acceptance criteria ≤ 2.0%)
Regorefenib	40 ppm	1	2271419	0.01
		2	2271652	
		3	2271987	
		4	2271410	
		5	2271762	
		6	2271854	

Table.3

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Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTRA DAY RSD (Acceptance criteria ≤ 2.0%)
Regorefenib	40	1	2271235	0.0204
		2	2271965	
		3	2272099	
		4	2272137	
		5	2272416	
		6	2272563	

TABLE.4

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.2 ppm dilution Peak was not clearly observed, based on which 0.02 ppm is considered as Limit of Detection and Limit of Quantification is 0.5 ppm.

Parameter	Measured Value
Limit of Quantification	0.5 ppm
Limit of Detection	0.2 ppm

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample (µg/ml)	Area
1	2271354
2	2271788
3	2272297
4	2272435
5	2272519
6	2272238
RSD	0.0196

Table.5

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Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	2271376	……
2	MP	MeoH :ACN	2272345	
		75:25	2271239	0.60
		35:65		0.17
3	PH	5.0	2271610	0.13
		5.4	2271369	0.69
4	WL	259 nm	2271438	1.11
		263 nm	2271982	0.28

Table.6

Recovery

Recovery test was performed at 3 different concentrations i.e. 30ppm, 40ppm, 50ppm. Results are given in table.7

% Recovery	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	20	10	30	29.8	99.33
	20	10	30	29.64	98.8
	20	10	30	29.75	99.16
100%	20	20	40	39.88	99.7
	20	20	40	39.67	99.175
	20	20	40	39.92	99.8
150%	20	30	50	49.68	99.36
	20	30	50	49.77	99.54
	20	30	50	49.81	99.62

Table.7

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Formulation Analysis

S.NO	Tablet	Dosage	Sample conc	Sample Estimated	% of Drug Estimated in Tablet
1	Stivarga	120 mg	40 ppm	39.82 ppm	99.55

Table.8: formulation results

CONCLUSION

The proposed method for the assay of Regorefenib in tablets is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. So the column will work efficiently.

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