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Research Article

METHOD DEVELOPMENT AND VALIDATION OF SUNITINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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Abstract

In the present work, a new validated HPLC method for quantitative determination of Sunitinib in capsule formulation was developed. The column was Phenomenex Luna C_{18} column (150 mm × 4.6 mm id; 5µm particle size) and the mobile phase was composed of Acetonitrile: Methanol: Water (70:20:10, v/v/v) with a flow rate 1 ml/min. Eluents were monitored by UV detector at 277 nm. Calibration curve was linear in the concentration range 3 – 15 µg/ml (R^2 value is 0.9993). The proposed method was successfully applied for the assay of Sunitinib in capsule formulation and validated as per ICH guidelines.

Keywords: Sunitinib, HPLC Method, UV Detector and ICH guidelines.

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INTRODUCTION

Sunitinib (Figure 1), chemically N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-2-oxo-1H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide [1]. It inhibit activation Hedgehog pathway. Sunitinib is primarily metabolized by CYP3A and is eliminated hepatically [2].

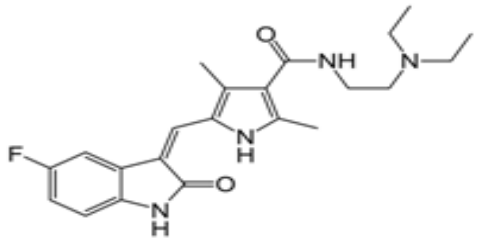

Fig. 1: Chemical structure of Sunitinib [3]

On literature survey, No method was reported for the estimation of Sunitinib. So we have developed a novel, simple, rapid, accurate, precise and highly sensitive RP-HPLC method for the estimation Sunitinib in capsule dosage form according to ICH guidelines. The developed method was validated as per ICH norms.[4-5]

MATERIALS AND METHODS

Materials and reagents

Pharmaceutical grade standard Sunitinib were obtained from Beijing mesochem technology co.ltd. (China). The pharmaceutical dosage form used in this study was Odomzo capsule contains equivalent to 200 mg of Sunitinib per capsule. All chemicals and reagents were of HPLC grade and were purchased from Sudhagar Biological and Chemicals Pvt.
Ltd., Chennai, India.

The instrument and chromatographic conditions

Shimadzu HPLC system (Shimadzu corporation Kyoto, Japan) consisted of a pump (LC - 20AD solvent deliver module, SPD-20A UV- Visible detector) run under Lab solutions software, with manual injecting facility programmed at 20 µL capacity per injection was used. The column used was Phenomenex Luna C18 (150 mm × 4.6 mm, 5.0 µm particle size). Different mobile phases were tested in order to find the best condition for separation of Sunitinib. The mobile phase contained Acetonitrile: Methanol: Water (70:20:10, v/v/v) and the flow rate was maintained at 1.0 ml/min. UV detection was carried out at 277 nm. The mobile phase and samples were filtered through a 0.45 µm membrane filter. Mobile phase was degassed by Sonica ultrasonic cleaner (model 2200 MH) prior to use.

Preparation of standard and sample solutions

Diluent

Mobile phase was used as the diluent.

Mobile phase

Acetonitrile: Methanol: Water (70:20:10, v/v/v) is programmed as RP HPLC method.

Preparation of standard stock solution

Accurately weighed quantity of 200 mg of Sunitinib API was transferred into 100 ml volumetric flask and dissolved in diluent and diluted up to mark. From this standard stock solution, 1.5ml was withdrawn and diluted to 100 ml using mobile phase to get working concentration of 30 μg/ml.

Preparation of working standard solution

From above standard stock solution of Sunitinib, 2ml of solution was taken into 10 ml volumetric flask and was made to the mark with the mobile phase to get 6 μg/ml of Sunitinib.

Preparation of sample stock solution

The average weight of 20 capsules was determined. Sample stock solution was prepared by dissolving capsule powder equivalent to 200 mg of Sunitinib was transferred to 100 ml volumetric flask. Then 60 ml diluent was added and sonicated for 10 mins to ensure complete solubilization of drug. After sonication, volume was made up to the mark with diluent. Filter the stock solution with whatman filter paper. Then, 1.5ml was withdrawn and diluted to 100 ml using mobile phase to get concentration of 30 μg/ml is a sample stock solution.

Preparation of sample solution

From above sample stock solution of Sunitinib, 2.0 ml was withdrawn and diluted to 10 ml using mobile phase to get concentration of 6 μg/ml is a sample solution.

Validation

The proposed methods were validated as per ICH guidelines.

Linearity

Different aliquots of 1.0 - 5.0 ml of standard stock solution was transferred into series of 10 ml volumetric flasks, separately and the volume was made up to the mark with mobile phase to get concentrations 3, 6, 9, 12 and 15 µg/ml, respectively.

Accuracy

To the preanalysed sample solution, a known amount of standard stock solution was added at different levels i.e. 50, 100 and 150%. The solutions were reanalyzed by proposed method.

Precision

The reproducibility of this method was determined by analyzing capsules at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision).

RESULTS AND DISSCUSION

Method development and optimization

The HPLC procedure was optimized with a view to develop a suitable LC method for the determination of Sunitinib in capsule dosage form. Initially, methanol and water in different ratios were tried. But Sunitinib gave broad peak, so acetonitrile was added and mixtures of acetonitrile, methanol and water in different ratios were tried. It was found that acetonitrile : methanol : water in ratio of 70 : 20 : 10 (v/v/v) gave acceptable retention times (3.473 min) with flow rate of 1.0 ml/min as shown in fig. 3 and also performed mobile phase black as shown in fig. 2.

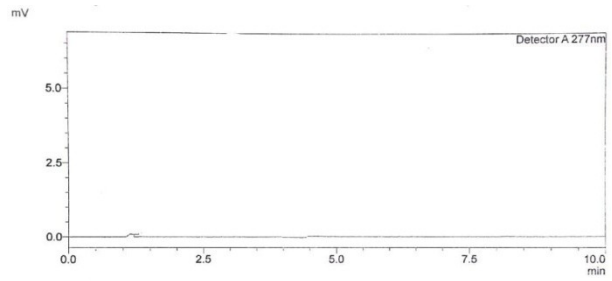

Fig. 2: Chromatogram of mobile phase blank.

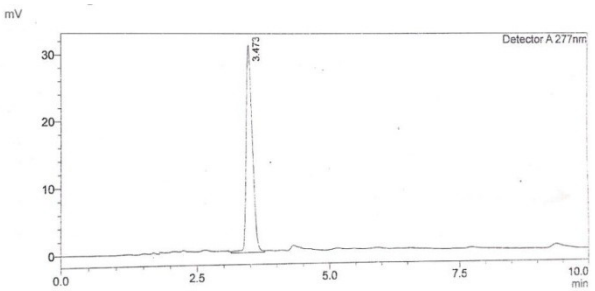

Fig. 3: Chromatogram of Sunitinib obtained at optimum chromatographic conditions.

Method validation

The described method has been validated which include parameters like system suitability, linearity, accuracy, precision, robustness, LOD (limit of detection) and LOQ (limit of quantification).

System suitability

System suitability and chromatographic parameters were validated such as tailing factor and theoretical plates was calculated. The results are given in table 1.

Table 1: System suitability parameters.

Parameters	Sunitinib	Recommended limits
Retention time	3.473	RSD ≤2
Peak area	279600	RSD ≤2
Tailing factor (T)	1.425	T <2
Theoretical plate (N)	4190	> 2000

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solution of Sunitinib at different concentration in the range of 3 -15 µg/ml with correlation coefficient (r^2) of 0.9993. Results are given in table 2.

Table 2: Linearity data for Sunitinib.

Drug	Concentration (µg/ml)	Area
Sunitinib	3	136076
	6	278130
	9	404007
	12	542038
	15	689438

Accuracy

Accuracy of the proposed method was determined by performing the recovery experiment. The recovery experiment was studied by adding known amount of standard Sunitinib to the pharmaceutical product and calculating the recovered standard amount. At 50%, 100% and 150% standard addition level mean recovery of Sunitinib found to be 100.08%, 100.23% and 99.61% respectively. The results of recovery experiment are given in table 3.

Table 3: Results of the accuracy study.

Accuracy Level	Amount of drug taken (mg)	Amount of drug spiked (mg)	Recovery %	RSD% (n=3)
50	200	100	100.08	0.1618
100	200	200	100.23	0.1591
150	200	300	99.61	0.5207

Precision

Precision was evaluated at the repeatability and intermediate precision levels. For repeatability analysis, six independent portions of a capsule dosage form were processed through the full analytical method and results was evaluated obtaining a % RSD value of 0.5207 as shown in table.4.

Table 4: Precision result of the proposed method.

Sample No.	Sunitinib	
	Peak area response	Assay (%)
1	278258	100.04
2	276809	99.52
3	276162	99.29
4	279600	100.52
5	277945	99.93
Average	277755	99.61
% RSD	0.4805	0.5207

Robustness

Robustness study was conducted by deliberate changes in mobile phase composition and flow rate, revealed that there was no significant variation in % assay as shown in table.5.

Table 5: Robustness study.

Percent assay of the drug	Mobile phase, Acetonitrile: Methanol: Water		Flow rate, ml/min	
Sunitinib	65 : 25 : 10 (v/v/v)	75 : 15 : 10 (v/v/v)	0.9	1.1
	100.32	100.44	99.96	100.12

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ was found to be 0.4089 (µg/ml) and 1.2269 (µg/ml) forSunitinib estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The results are given in table.6.

Table 6: LOD and LOQ data for Sunitinib.

Drug	LOD	LOQ
Sunitinib	0.4089 (µg/ml)	1.2269(µg/ml)

CONCLUSION

The HPLC method was developed and validated according to ICH guidelines and was applied for the determination of Sunitinib in capsule formulation. The result obtained from validation studies revealed that, the developed method was found to be rapid, simple, accurate, precise, specific, selective and economical. Hence, this method can easily and conveniently adopt for routine analysis of Sunitinib in pure and its capsule dosage form.

AUTHOR CONTRIBUTION

All aythors Contributed Equally.

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CONFLICT OF INTREST

No Conflict of Intrest

REFERENCES

1. https://www.drugbank.ca/drugs/DB09143
2. https://en.wikipedia.org/wiki/Sunitinib
3. https://www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:90863
4. Code Q2A: Text on Validation of Analytical Procedure Methodology Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, 2005.
5. Code Q2B: Text on Validation of Analytical Procedure Methodology Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, 1994.