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Stress Degradation Studies And Development Of Stability Indicating Assay Method For Estimation Of Sorafenib In Bulk And Its Formulation

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ABSTRACT: -

A sensitive high-performance liquid chromatographic (HPLC) method were developed and validated for the estimation of Sorafenib tosylate in bulk and pharmaceutical formulations. The chromatographic separation was achieved by RP-HPLC using a mixture of Acetonitrile: Methanol (45:55) as the mobile phase, a C18 column & at 263 nm with flow rate 1ml/min. The linear and reproducible calibration curve over the range was 2–20 µg/ml. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of Sorafenib tosylate in pharmaceutical formulations. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming. The method showed adequate precision with a relative standard deviation (RSD) smaller than 3%. The accuracy was analyzed by adding a standard drug and good recovery values were obtained for all drug concentration used. Degradation studies were carried out under conditions of Alkali Degradation, Acid Degradation, dry heat Degradation, Peroxide Degradation, and UV light and the drug substances were degraded in all conditions. Force degradation studies shows all the degradant peak obtained during degradation were well resolved from main peak of the drugs. The analytical procedure is reliable and offers not only advantages in terms of speed but also met the regulatory requirements for specificity, Linearity, LOD, LOQ, Precision, accuracy

KEYWORDS: Sorafenib tosylate, Marketed formulation, Spectrophotometer, HPLC.

1. INTRODUCTION

Sorafenib tosylate (SORA) is a bi-aryl urea and an oral multikinase inhibitor. It targets cell surface tyrosine kinase receptors and downstream intracellular kinases that are implicated in tumour cell proliferation and tumour angiogenesis. Sorafenib is used to treat late-stage kidney cancer (advanced renal cell carcinoma), liver cancer (hepatocellular carcinoma) that cannot be treated by surgery, and differentiated thyroid cancer that has come back or spread to other parts of your body. Sorafenib is an antineoplastic (cancer) agent. Chemically it is 4-[4-[[4-chloro-3-(trifluoromethyl) phenyl] carbamoylamino] phenoxy] -*N*-methylpyridine-2-carboxamide.

High-performance liquid chromatographic (HPLC) is the most frequently applied technique in the determination of drugs in biological fluids and dosage forms. We believe that the availability of this new method, with increased simplicity, sensitivity and selectivity, will be very useful for the determination of Sorafenib (SORA) in raw material and pharmaceutical preparations. This gradient HPLC method uses a simple mobile phase and does not require complicated sample preparation. The aim of this study was to develop a simple, rapid and reproducible reversed-phase HPLC method.

The ICH guideline for stability testing of new drug substances and products requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions to determine the stability of the molecule. Stress testing is a critical component of drug development. By generating key stress-testing samples (i.e. degraded samples stressed under various conditions), predictive to a drug company in terms of time and money. In addition, stress testing can help in the selection of more stable drug substance salt forms and drug formulations. Stress testing also becoming increasingly important in testing new molecules. Methods developed by stress testing and stability information gained from those methods can have a significant effect on the actual compound selected for development.

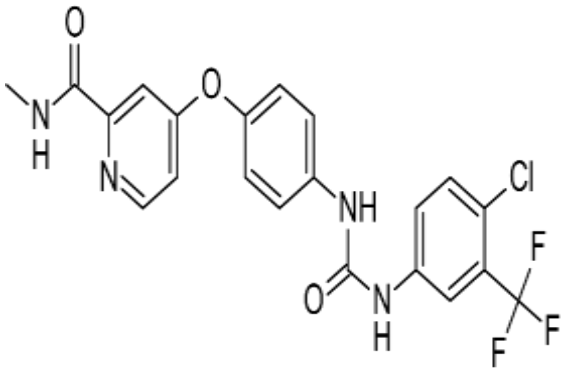
In this studied we tried to develop stability indicating the HPLC technique for SORA in pharmaceutical dosage form. The present work describes a simple, stability indicating HPLC method for the determination of SORA in pharmaceutical dosage form according to ICH guidelines.

2. EXPERIMENTAL

2.1 Materials

SORA was supplied by Mac- Chem Products India Pvt Ltd and was used without further purification. Sodium hydroxide was purchased from Molychem (Mumbai). Hydrochloric acid and hydrogen peroxide was procured from LOBA Chemie Pvt. Ltd. (Mumbai). HPLC grade methanol and acetonitrile was purchased from S. D. Fine-chem Ltd. (Mumbai) whereas HPLC grade water was purchased from Merck Ltd. All other chemicals were of analytical reagent grade. Sorafenib marketed (manufactured Sun Pharma Lab. Ltd India.) were purchased from Local market.

2.2 Chemical structure :



2.3 Instrumentation

The HPLC system consisting of Thermo Separation Quaternary Gradient HPLC pump Spectra System P4000 with PDA detector of Spectra System, manual rheodyne injection system, the software was an Data ace software version 6.1. The chromatographic separation was performed using Grace C_{18} (250mm × 4.6 mm i.d., 5mm particle size) Separation was achieved using a mobile phase consisting of Acetonitrile: Methanol in the ratio (45:55) at a flow rate of 1ml/min and UV detection at 263 nm. The column was maintained at ambient temperature with injection volume of 20 µl. The mobile phase was filtered through 0.45 µm Chrom Tech Nylon-66 filter and degassed in ultrasonic bath prior to use. A blank chromatogram was recorded before the studies. Quantization of result was performed using peak area counts.

2.4 Standard preparation

Stock solution of SORA was prepared. Accurately weighed quantity 5 mg of was dissolved in acetonitrile and volume was made up to 25 ml mark (200 µg/ml). The stock standard solution was diluted further to get final concentration of about 10 µg/ml. then various trial are taken & mobile phase finalized where proper resolution of both the drug were seen. This was found that the sample preparation in mobile phase gives sharp resolution hence all sample were prepared in mobile phase. The stock solution was prepare in mobile phase of 100µg/ml.

2.5 System suitability test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

2.6 Application of proposed method for estimation of SORA in laboratory sample

Three different laboratory mixtures of SORA were prepared by appropriately weighing the quantities of drug samples so as to get the concentration of 50 µg/ml of SORA. The peak area of standard laboratory sample and Test laboratory sample was compared to obtain the concentration.

2.7 Application of proposed method for estimation of SORA in formulation:

For the estimation of drug from commercial formulation twenty tablets were weighed accurately. The average weight was determined, finely powdered and powder equivalent to 10 mg of drug was transferred. The test and sample solution prepared, the solution was filtered through Whatman filter paper no. 41. Further dilution was made to get final

concentration of 50 µg/ml of SORA. Equal volume (50µg /ml) of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and response i.e. peak area were measured.

3 RESULT & DISCUSSION:

3.1 Preparation of Calibration Curve: -

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 2-20 µg/ml of both drug solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area depicted in Fig. No.2.

3.2 Method Validation

3.2.1 Specificity (Selectivity)

Specificity was measured as ability of the proposed method to obtain well separated peak for SORA without any interference from component of matrix. The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix. Mean retention time for – SORA – 3.888

3.2.2. Accuracy and precision

It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded in Table No. 3 Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

3.2.3 Ruggedness:

The studies of ruggedness were carried out under two different conditions-

a) Days
b) Analyst.

a) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 4

b) Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

3.3 Force degradation studies

Specificity of the method was determined by calculating percent amount of possible degradation products produced during the force degradation study. The stress conditions applied for degradation study involved acid, base, neutral, sunlight, thermal, UV photolysis, and oxidative degradation for find out the stability nature of the drug. The degradation samples were prepared by taking suitable aliquots of the drug and drug product solution and then undertaking the respective stress testing procedures for each solution. After the fixed time period the treated drug solutions were diluted with solvent results are mentioned in the table no.7.

4 CONCLUSION

Forced degradation studies of different condition shows all degradants were well resolved from main peak also able to quantify the SORA in the presence of excipients as well as degradants proves method is found to be stability indicating. Hence proposed method adopted for routine analysis in bulk and dosage form.

Tables:

Table No1 . : Summary of system suitability test results

Sr.no.	Parameter	SORA
1.	Peak area	541983.5
2.	Retention time (min)	3.842
3.	Tailing Factor (T)	1.12
4.	No. of Theoretical plates (N)	6967.84

***Results are mean of five replicates**

Table No. 2: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method

Sr. no.	Sample	Statistical data	% Estimation	% Recovery
			SORA	SORA
1.	Standard Laboratory mixture	Mean	99.86	-
		S.D.	0.71	-
		C.V.	0.007	-
2.	Marketed formulation	Mean	99.95	99.33
		S.D.	0.78	0.48
		C.V.	0.61	0.23

Table No 3: Summary of validation parameters for the proposed method

Validation Parameters	SORA
Linearity µg mL-1	2-20
Accuracy mean	99.33
Precision (% RSD)	0.82

Table No 4: Summary of Ruggedness by RP-HPLC method

Parameter	Statistical data	% Estimation
		SORA
Interday	Mean	100.17
	S.D.	1.21
	C.V.	1.20
Intraday	Mean	99.53
	S.D.	0.93
	C.V.	0.94
Different analyst	Mean	99.89
	S.D.	0.2748
	C.V.	0.2750

Table No.5: Result and statistical data of Different analyst study

Sr. No	**% Label claim**	
	ANALYST I	**ANALYST II**
	SORA	**SORA**
1	99.85	100.3
2	100.15	99.77
3	99.66	99.61
4	100.2	99.8
5	99.60	100.18
Mean	99.89	99.93
± S.D	0.2748	0.2933
C.V	0.2750	0.294

Table No.6: Observations of Linearity and range study for SORA.

Sr.No.	**%Label claim**	**Peak area**
		SORA
1	80	440789
2	90	495551
3	100	550612
4	110	600673
5	120	661834

Table No 7: Stressed Degradation studies of SORA

Sr. No.	**Injection**	**% Degradation**	**Purity Angle**	**Purity Threshold**	**Purity Flag**
1	Acid Degradation	6.33	0.076	0.249	No
2	Base Degradation	12.22	1.027	0.911	No
3	Peroxide Degradation	9.56	0.123	0.490	No
4	Thermal Degradation	4.55	0.090	0.149	No
5	UV Degradation	1.37	0.815	0.265	No

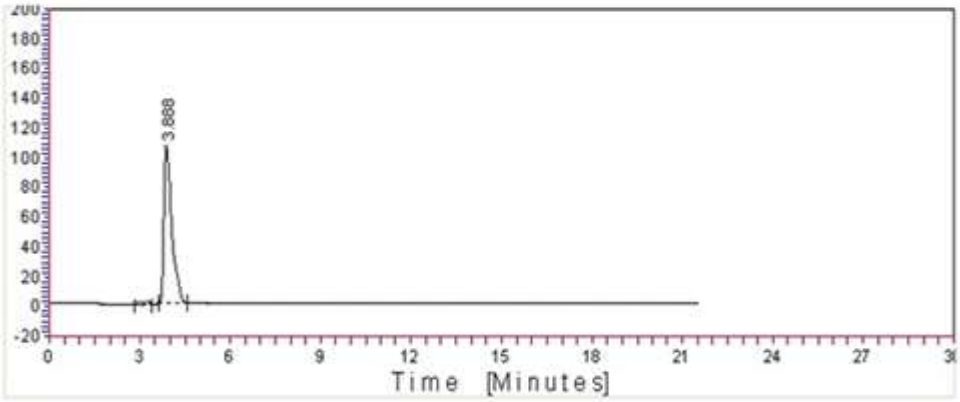


Fig. No.1: Chromatogram obtained by formulation of SORA

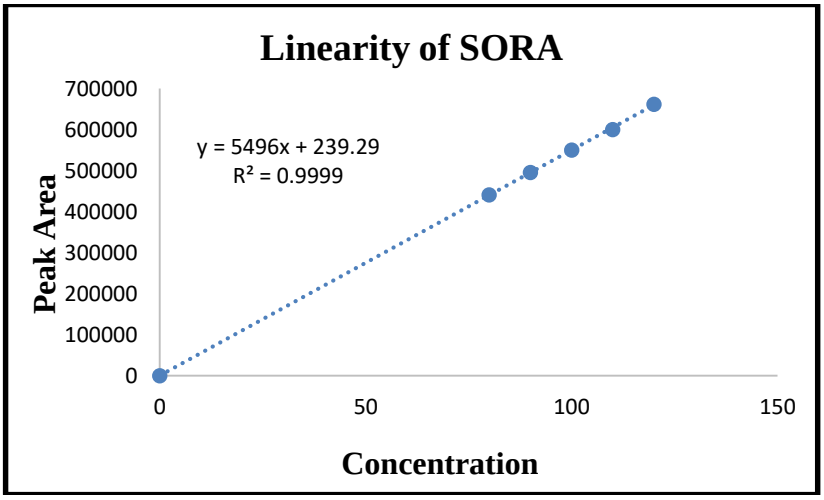


Fig no.2: linearity and range study for SORA

5 REFERENCES

1. Sethi. P.D., HPLC, Quantitative analysis of pharmaceutical formulation, CBS Publisher and Distributor, New Delhi, preface, 101
2. Beckett AS., Stenlake JB., "Practical Pharmaceutical chemistry", fourth edition, volume 2, CBS Publisher and Distributor, New Delhi, 1997; 1-85.
3. https://en.wikipedia.org/wiki/Sorafenib
4. M. Sudheer1, A. B. N. Nageswara Rao, D. Hari Hara Theja, M. Siva Prakash, Development of Stability Indicating RP-HPLC Method for Simultaneous Determination of Azithromycin and Ambroxol HCl (SR) in the Tablet Formulation. Der Pharmacia Lettre, 2012, 4 (3):803-810
5. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization, IFPMA, Geneva, 2003
6. Indian Pharmacopoeia, Government of india ministry of health & family welfare, published by the Indian Pharmacopoeia commission, Ghaziabad, 2014; vol. III: 2774.
7. Jayaprakash R, Natesan S. Optimization of stability indicating RP-HPLC method for the estimation of an anti-cancer drug Sorafenib Tosylate in pure and pharmaceutical dosage form. Int. J. Of Pharmacy and Analytical Research, 2017; 6(1): 141-152.
8. Database of FDA Approval for Sorafenib Tosylate, Compilation prepared by National Cancer Institute, https://www.cancer.gov/aboutcancer/treatment/drugs/fda-sorafenib-tosylate date of access: 18 March 2018.
9. Database of Pubchem open chemistry, Compilation prepared by U. S. National Library of Medicine, https://pubchem.ncbi.nlm.nih.gov/compound/Sorafe nib_tosylate#section =Top date of access: 18 march 2018.
10. ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products, International Council for Harmonisation Geneva. ⟨http://www.ich.org/products/ guidelines /quality/ article/ quality-guidelines/html), 2003

11. Heinz WJ, Kahle K, Beyersdorf A, Schirmer D, Lenker U, Keller D, Langmann P, Klinker H. Highperformance liquid chromatographic method for the determination of sorafenib in human serum and peritoneal Xuid. Cancer Chemother Pharmacol, 2011; 68: 239–245.
12. Kalaichelvi R, Jayachandran E. Quantitative Estimation of Sorafenib Tosylate Its Pure Form and in Its Tablet Formulation by RP-HPLC Method. Journal of Chemistry, 2013: 1-3.
13. Damle MC*, Kale S. Development and Validation of Stability-Indicating HPTLC Method for Estimation of Sorafenib tosylate. European Journal of Biomedical and Pharmaceutical Sciences, 2017; 4(6): 432-438.
14. ICH guidelines, Q2 (R1): Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization Geneva. ⟨http://www.ich.org/products/ guidelines/quality/article/quality guidelines/html), 2005
15. Michael W. Dong," Modern HPLC for practicing scientist" A John Wiley & Sons, Inc., Publication, Page no. 194,217.
16. Jeffery GH., Basset J., Mendham J., Denney RC., "Vogel's textbook of quantitative analysis", 5th Edn., Longman Scientific and Technical, 1999: Page no. 10-11.
17. Skoog, DA., West DM., Holler FJ., "Analytical chemistry –An Introduction", 6th Edn., Saunder college publishing, 1994: Page no 3.
18. Sharma BK., "Instrumental methods of chemical analysis", 23rd Edn., Goel publishing House, Meerut, 2004: Page no7-8.
19. Beckett AS., Stenlake JB., "Practical Pharmaceutical chemistry", 4th Edn, vol 2, CBS Publisher and Distributor, New Delhi, 1997: Page no 1-85.
20. Christianah MA., Pui-Kai L., "Analytical Profile of Drug Substances" Edi. By Klaus Florey, Page no 124-141.
21. Beckett, A.H., Stenlake. J.B., "Practical Pharmaceutical chemistry", 4th Edn., Part –II, CBS Publishers and Distributors, New Delhi, Page no 278-282.
22. Ahuja S., Scypinski S., "Handbook of modern pharmaceutical analysis", Vol.3, Academic press,2001: Page no 349.
23. Christen GD., Analytical Chemistry, fifth ed., John Wiley & Sons, Inc.2001: Page no 505
24. Illet and Ripper, "Selectivity optimization in HPLC", In; Brown, R., Phyllis, Eligrushka, Advances in chromatography, 39, Marcel Dekker, Inc.,1998: Page no 264-65.
25. Sethi PD., PLC-Quantitative analysis of pharmaceutical formulations", CBS publishers and Distributors, New Delhi, 2001: Page no 11.
26. Sethi PD., HPLC-Quantitative analysis of pharmaceutical formulations", CBS publishers and Distributors, New Delhi,200 Page no 1-5.