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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF SOTORASIB IN ITS PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A precise approach based on RP-HPLC methodology was developed for Sotorasib estimation. Using stationary phase BDS 150mm x 4.6 mm, 5m, mobile phase 0.01N, the chromatographic conditions employed are Acetonitrile in a 70:30 ratio maintained a flow rate of 1 ml/min, a detection wave length of 268 nm, a column temperature set to 30 oC, and a mobile phase diluent ratio. Conditions were settled as best approach. By injecting the standard six times, system suitability parameters were investigated and results fell well under the acceptance requirements. Between 25% and 150% levels, linearity studies revealed an R2 value of 0. 999. Repeatability determined to be 0.1; intermediate precision found to be 0.3.LOD and LOQ are respectively 0.28µg/ml and 0.83µg/ml. Using aforesaid approach test of marketed formulation resulted in 99.54% presence. Sotorasib's degradation investigations revealed that within the permissible range and in every condition's purity threshold was more than purity angle. The whole length approach was not followed; so, this technique may be applied for regular Sotorasib analysis.

Key Words: Sotorasib, Method development, Validation, RP-HPLC.

INTRODUCTION[1-10]

Sotorasib is a groundbreaking small-molecule inhibitor developed to target the KRAS^G12C^ mutation, one of the most prevalent oncogenic drivers in several cancers, including non-small cell lung cancer (NSCLC), colorectal cancer (CRC), and others. KRAS mutations have long been considered "undruggable" due to the protein's smooth surface and the lack of known binding pockets. Sotorasib's discovery marks a significant milestone in oncology, offering the first direct approach to targeting KRAS mutations.

KRAS, a member of the RAS family of GTPases, plays a central role in cell signaling pathways that regulate proliferation, differentiation, and survival. The KRAS^G12C^ mutation leads to a constitutively active KRAS protein that drives continuous oncogenic signaling, which contributes to cancer progression. Sotorasib selectively and irreversibly binds to the mutant cysteine residue at position 12 (G12C) of the KRAS protein, locking it in its inactive GDP-bound form, thereby blocking downstream signaling in the RAS-MAPK pathway.

Sotorasib was approved by the U.S. Food and Drug Administration (FDA) in May 2021 under the trade name Lumakras, making it the first FDA-approved therapy to target KRAS^G12C^. The drug is currently indicated for the treatment of patients with locally advanced or metastatic NSCLC harboring the KRAS^G12C^ mutation, following at least one prior systemic therapy. This approval followed the success of the Phase I/II CodeBreaK 100 trial, which demonstrated durable anticancer activity and manageable safety in heavily pre-treated patients.

In clinical trials, sotorasib has shown promising efficacy, particularly in patients with NSCLC. The CodeBreaK 100 trial reported an overall response rate (ORR) of 37.1%, with a median progression-free survival (PFS) of

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6.8 months in patients with advanced NSCLC. The drug is administered orally once daily, making it convenient for patients.

Beyond NSCLC, sotorasib is being studied in other KRAS^G12C^-mutant cancers, including colorectal cancer, pancreatic cancer, and solid tumors. However, efficacy in colorectal cancer has been more modest, suggesting that combination therapies with agents such as EGFR inhibitors or immune checkpoint inhibitors may be needed to enhance responses in this population.

As with many targeted therapies, resistance to sotorasib can develop. Preclinical and clinical studies have shown that secondary KRAS mutations or activation of alternative pathways can lead to treatment resistance. Ongoing research is focused on understanding these resistance mechanisms and exploring combination strategies to overcome them.

In conclusion, sotorasib represents a major advancement in targeted cancer therapy, providing the first effective treatment for KRAS^G12C^-mutant cancers, particularly NSCLC. It has opened the door for future therapies aimed at KRAS mutations and serves as a foundation for ongoing research to expand its use across different cancer types and improve therapeutic outcomes through combination approaches.

Analytical Background[11]

Sotorasib is an experimental KRAS inhibitor being investigated for the treatment of KRAS G12C mutant non-small cell lung cancer, colorectal cancer, and appendix cancer. It is chemically known as 6-fluoro-7-(2-fluoro-6-hydroxyphenyl)-1-[4-methyl-2-(propan-2-yl) pyridine-3-yl]-4-[(2S)-2-methyl-4-(prop-2-enoyl)piperazin-1-yl]-1H,2H-pyrido[2,3-d] pyrimidin -2-one

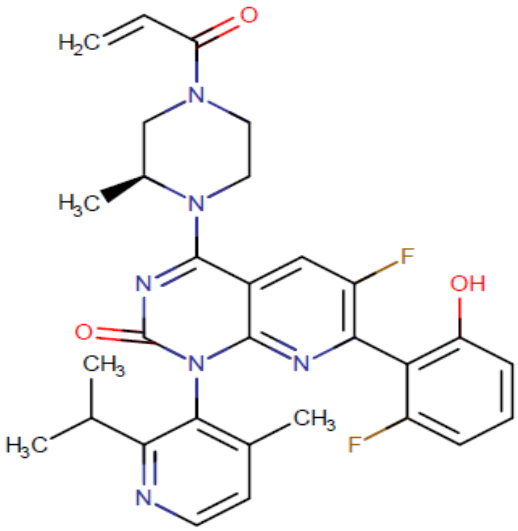

Figure 1 Structure of Sotorasib

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Sotorasib, In the review of literature, more economical methods were observed12-16, hence a simple, cost-effective stability-indicating simultaneous estimation of Sotorasib by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS:

Sotorasib pure drug (API), Sotorasib formulation (Sotoxen), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions:

Mobile phase	0.01N Ammonium acetate : Acetonitrile (60:40 v/v)
Flow rate	1 ml/min
Column	BDS C18 (4.6 x 150mm, 5µm)
wave length	268 nm
Column temperature	30°C
Injection volume	10µL
Run time	5.0 min

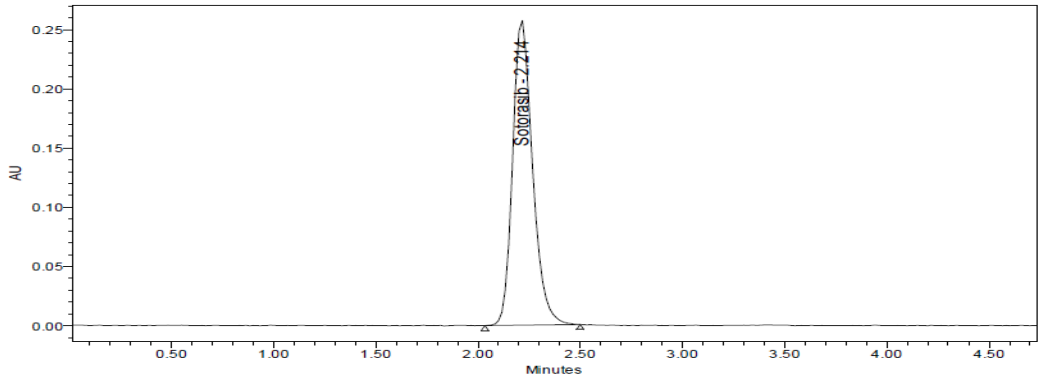

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 12mg of Sotorasib transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (24µg/ml of Sotorasib)

Preparation of Standard working solutions (100% solution): 1ml of Sotorasib from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (13.4µg/ml of Sotorasib)

Preparation of Sample stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 100ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (24µg/ml of Sotorasib)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Sotorasib (24 ppm) and the solution were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2

Table: 2 System suitability parameters for Sotorasib

S no	Sotorasib		
Inj	**RT(min)**	**USP Plate Count**	**Tailing**
1	2.224	2505	1.25
2	2.227	2498	1.23
3	2.229	2575	1.23
4	2.230	2617	1.23
5	2.232	2707	1.22
6	2.239	2521	1.24

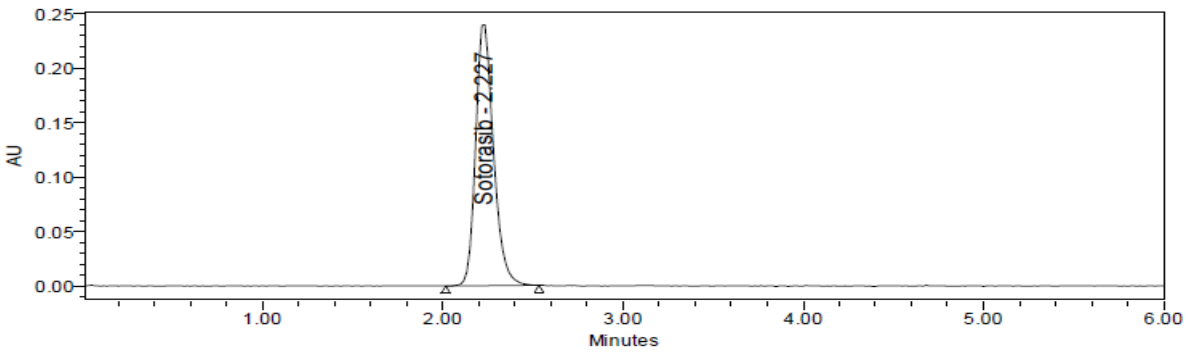

Figure 3: System Suitability Chromatogram of Sotorasib

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Sotorasib	2.214	1745716	2568.9	1.2

Specificity:

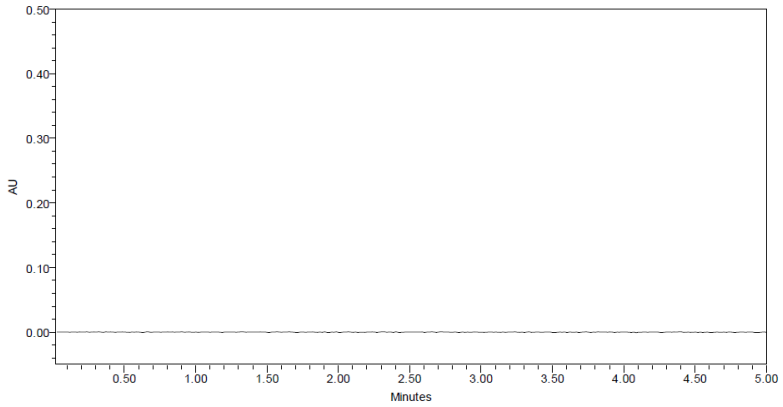

Figure 4 Chromatogram of blank.

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5

Table 4: Forced degradation conditions for Sotorasib

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxidation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Condition	% Drug Un Degraded	% Drug Degraded
Acid	99.08	0.92
Base	93.36	6.64
Oxidation	98.41	1.59
Thermal	99.17	0.83
Photolytic	98.95	1.05
Hydrolytic	98.90	1.10

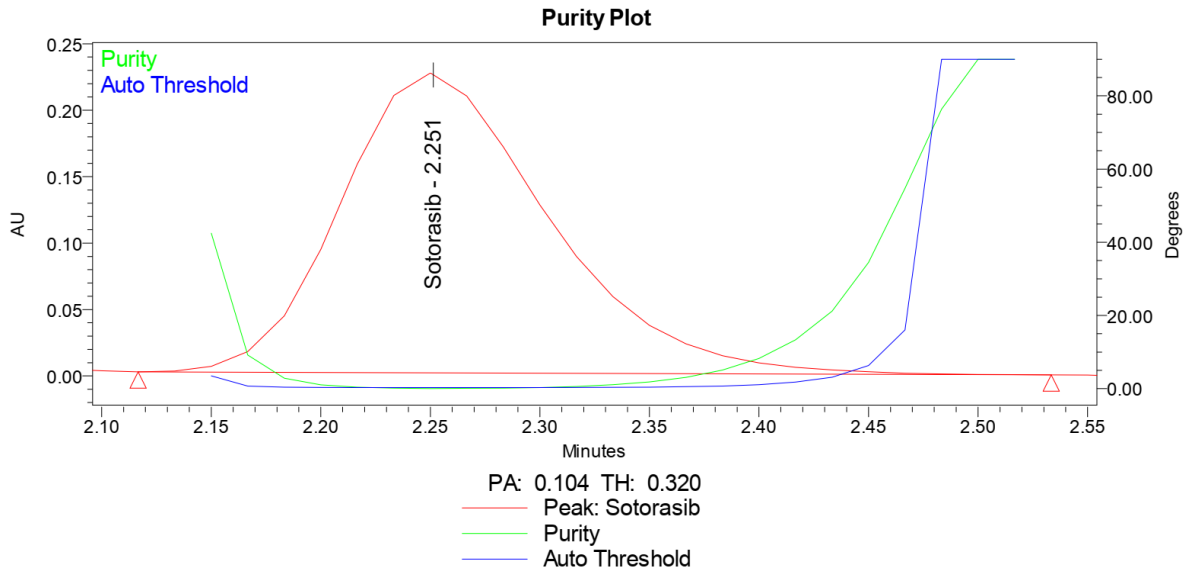

Figure 5: Purity Plot of Acid

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Sotorasib are listed in Table 6.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.140
LOQ	0.424

Linearity: The linearity of the method was demonstrated for Sotorasib by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Sotorasib was 0.999. This indicates good linearity

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 6

Table 7: Calibration data of Sotorasib

Sotorasib	
Conc (µg/mL)	**Peak area**
0	0
6	388872
12	828827
18	1239497
24	1612581
30	2012377
36	2393954

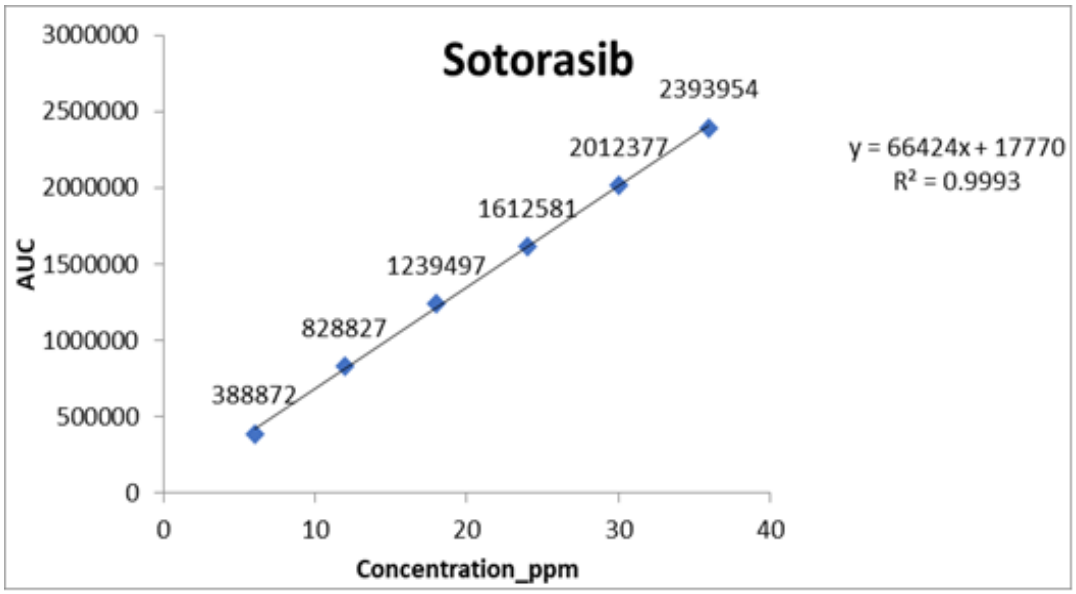

Figure 6: Calibration curve of Sotorasib

Table 8: regression data

Parameter	Metformin
Conc range (µg/mL)	6-36µg/ml
Regression Equation	y = 6642x + 17770
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Sotorasib at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9

Table 9 Accuracy table of Sotorasib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	12	12.00	100.01
	12	12.16	101.32
	12	12.00	99.98
100%	24	24.19	100.77
	24	24.13	100.54
	24	24.14	100.58
150%	36	36.26	100.73
	36	36.41	101.13
	36	36.33	100.92
Mean % recovery			**100.66**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Sotorasib. Results of peak area are summarized in Table 10

Table 10 System precision table of Sotorasib

S. No	Area of Sotorasib
1.	1651542
2.	1652651
3.	1656450
4.	1645821
5.	1652723
6.	1652093
Mean	**1651880**
S.D	**3436.9**
%RSD	**0.2**

Method Precision: The precision of the method was determined by analyzing a sample of Sotorasib). Data obtained is summarized in Table 11

Table 11 Repeatability table of Sotorasib

S. No	Area of Sotorasib
1.	1647208
2.	1646907
3.	1644783
4.	1651256
5.	1645671
6.	1649307
Mean	**1647522**
S.D	**2388.9**
%RSD	**0.1**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12

Table 12 Intermediate precision table of Sotorasib

S. No	Area of Sotorasib
1.	1643673
2.	1642438
3.	1635471
4.	1640802
5.	1633320
6.	1635465
Mean	**1638528**
S.D	**4307.7**
%RSD	**0.3**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Sotorasib

Condition	%RSD of Sotorasib
Flow rate (-) 0.9ml/min	0.4
Flow rate (+) 1.1ml/min	0.4
Mobile phase (-) 55B:45A	0.2
Mobile phase (+) 65B:35A	0.2
Temperature (-) 27°C	0.5
Temperature (+) 33°C	0.4

Assay data: -

Sotoxen Tablet bearing the label claims Sotorasib 1.34 mg. Assay was performed with the above formulation. Average % Assay for Sotorasib obtained was 99.54%. Assay data shown in table no 14.

Figure 7: Sotorasib Marketed Drug

Formula to calculate assay:

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{10}{5} \times \frac{P}{100} \times \frac{FV}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 14: Assay Data of Sotorasib

S.no	Standard Area	Sample area	% Assay
1	1651542	1647208	99.52
2	1652651	1646907	99.50
3	1656450	1644783	99.37
4	1645821	1651256	99.76
5	1652723	1645671	99.42
6	1652093	1649307	99.64
Avg	**1651880**	**1647522**	**99.54**
Stdev	**3436.9**	**2388.9**	**0.1443**
%RSD	**0.2**	**0.1**	**0.14**

CONCLUSION

The Sotorasib HPLC study findings reveal that this technique can accurately gauge the concentration and purity of the drug. Because it can be repeatedly utilised with crisp peak resolutions and uniform retention periods, this approach is excellent for both pharmacokinetic research and frequent quality control. Making sure Sotorasib performs as expected and is safe for medical usage as well as confirming its chemical composition depend on HPLC.

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