

## STABILITY INDICATING AND COST-EFFECTIVE ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SOTORASIB BY USING RP-HPLC

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### ABSTRACT

**Objective:** The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredient of Sotorasib.

**Methods:** A simple, selective, validated and well-defined stability that shows isocratic RP-HPLC methodology for the quantitative determination of Sotorasib. The chromatographic strategy utilized symmetry C<sub>18</sub> column of dimensions 150x4.6 mm, 3.5 μ, using isocratic elution with a mobile phase of acetonitrile and 0.1% orthophosphoric acid (70:30). A flow rate of 1 ml/min and a detector wavelength of 221 nm utilizing the PDA detector were given in the instrumental settings. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

**Results:** LOD and LOQ for the active ingredient were established with respect to test concentration. The calibration chart plotted was linear with a regression coefficient of R<sup>2</sup>>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

**Conclusion:** The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of the selected drug.

**Keywords:** Sotorasib, RP-HPLC, Development, Validation, Stability

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### INTRODUCTION

Sotorasib, sold under the brand name Lumakras is an anti-cancer medication [1, 2] used to treat non-small-cell lung cancer (NSCLC) [3, 4]. It targets a specific mutation, G12C, in the protein KRAS [5, 6], which is responsible for various forms of cancer [7]. The most common side effects include diarrhea [8], musculoskeletal pain [9], nausea [10], fatigue [11, 12], and liver damage [13] and cough [14]. Sotorasib is an inhibitor of the RAS GTPase [15, 16] family. Sotorasib is the first approved targeted therapy for tumors [17] with any KRAS mutation, which accounts for approximately 25% of mutations in non-small cell lung cancers. KRAS G12C mutations represent about 13% of mutations in non-small cell lung cancers. Because the G12C KRAS mutation is relatively common in some cancer types, 14% of non-small-cell lung cancer adenocarcinoma [18] patients and 5% of colorectal cancer [19, 20] patients, and sotorasib is the first drug candidate to target this mutation, there have been high expectations for the drug. The Food and Drug Administration [21] has granted a fast track designation to sotorasib for the treatment of metastatic non-small-cell lung carcinoma with the G12C KRAS mutation. Researchers evaluated the efficacy of sotorasib in a study of 124 participants with locally advanced or metastatic KRAS G12C-mutated non-small cell lung cancer with disease progression after receiving an immune checkpoint inhibitor [22, 23] and/or platinum-based chemotherapy [24, 25]. The major outcomes measured were objective response rate (proportion of participants whose tumor is destroyed or reduced) and duration of response. The objective response rate was 36% and 58% of those participants had a duration of response of six months or longer. The aim of the study is to estimate the pharma ingredient Sotorasib by using RP-HPLC.

### MATERIALS AND METHODS

#### Chemicals

Acetonitrile (HPLC-grade), orthophosphoric acid, water was purchased from Merck India Ltd, Mumbai, India. API of Sotorasib standard was procured from Glenmark, Mumbai.

#### The instrumentation

Waters alliance liquid chromatography (model 2695) was monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study [26].

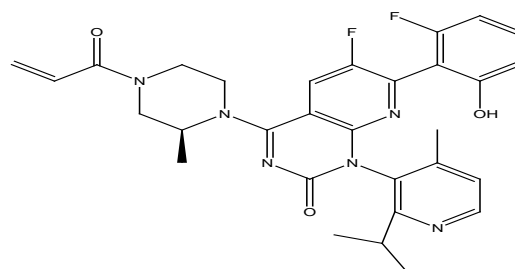


Fig. 1: Structure of sotorasib

#### Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic mode were tested. However, the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention time. Finally 0.1% orthophosphoric acid buffer and acetonitrile with isocratic elution was selected because it results in a greater response of active pharmacy ingredient. During the optimization of the method various stationary phases such as C<sub>8</sub>, C<sub>18</sub> and amino, phenyl columns were tested. From these trials the peak shape was relatively good with Symmetry C<sub>18</sub> column of 150 x 4.6 mm, 3.5 μ with a PDA detector. The mobile phase flow rate has been done at 221 nm in order to obtain enough sensitivity. By using above conditions we get retention time of Sotorasib was about 2.271 min with a tailing factor of 1.01. The

number of theoretical plates for Sotorasib was 4526, which indicate the column's successful output the % RSD for six replicate injections was around 0.59%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Till today there are no HPLC methods were reported in the literature, but only few methods are developed in the analysis of Sotorasib. Hence we developed method for the quantification of Sotorasib. The developed HPLC method was utilized for the estimation of the drug by *in vitro* method.

#### Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [27-29].

#### Preparation of buffer

1 ml of orthophosphoric acid (OPA) was dissolved in 1 litre of HPLC grade water and filtered through 0.45  $\mu$  filter paper.

#### Chromatographic conditions

The HPLC analysis was performed on a reverse-phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% OPA and Symmetry C<sub>18</sub> (150x4.6 mm, 3.5  $\mu$ ) column with a flow rate of 1 ml/min.

#### Diluent

Mobile phase was used as a diluent.

#### Preparation of the standard stock solution

For standard stock solution preparation, 70 ml of diluents was added to 100 mg of Sotorasib (taken in a 100 ml volumetric flask) and sonicated for 10 min to fully dissolve the contents and then makeup to the mark with diluent.

#### Preparation of Standard solution

1 ml of solution is drawn from the above normal stock solution into a 10 ml volumetric flask and diluted up to the level.

### RESULTS AND DISCUSSION

The main analytical challenge during the development of a new method was to separate active Pharma ingredients. In order to provide good performance, the chromatographic conditions were optimized.

#### System suitability

In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1 and the standard chromatogram was shown in fig. 2 [30].

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Sotorasib
USP Plate Count	NLT 2000	4526
USP Tailing	NMT 2.0	1.01
USP Resolution	NLT 2.0	-
% RSD	NMT 2.0	0.59

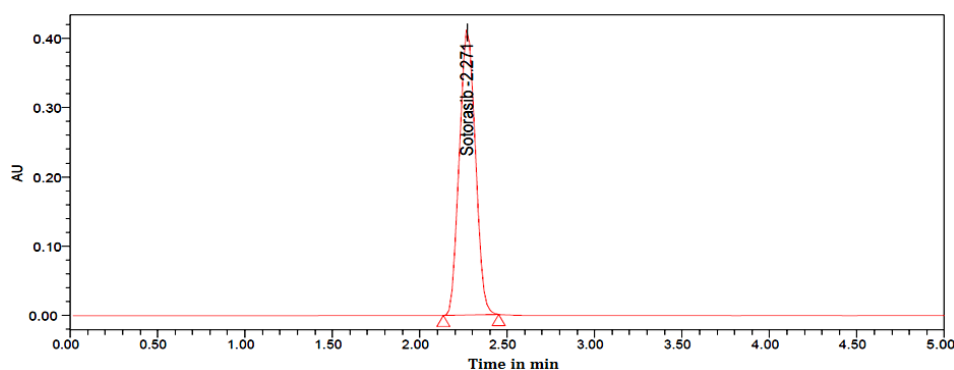


Fig. 2: Chromatogram of standard

#### Specificity

In this test method placebo and standard solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific [31].

#### Linearity

The area of the linearity peak versus different concentrations has been evaluated for sotorasib, as 10, 25, 50, 100, 125, 150 percent dilutions [32], respectively. Linearity was performed in the range of 10-150 $\mu$ g/ml of sotorasib. The correlation coefficient achieved greater than 0.999 for all.

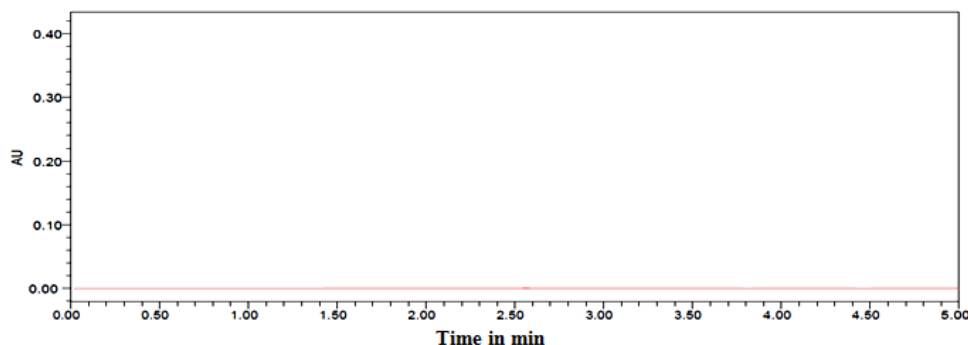


Fig. 3: Chromatogram of blank

Table 2: Linearity of sotorasib

S. No.	Conc µg/ml	Sotorasib area count
1	10.00	326971
2	25.00	731459
3	50.00	1347935
4	100.00	2619390
5	125.00	3263458
6	150.00	3946340
Correl coef		0.99982
Slope		25884.83
intercept		46933.12

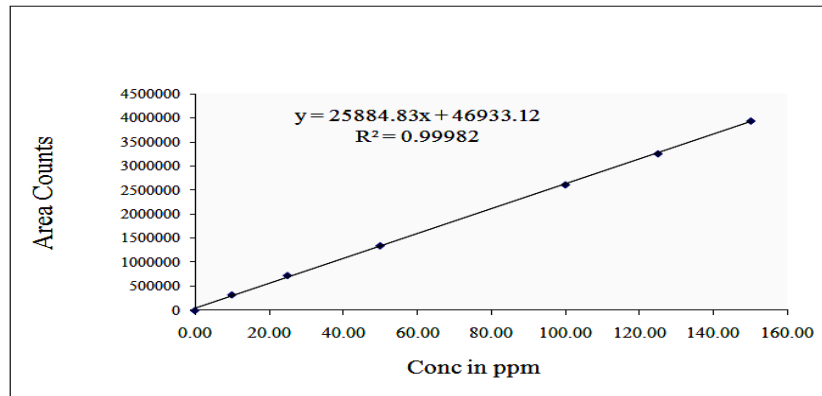


Fig. 4: Calibration plot of sotorasib

**Accuracy**

Three kinds of concentration levels of 50, 100, and 150 percent at a specified limit were used in this process to assess the accuracy of this particular method. The developed method was found to be highly accurate and reliable. The recovery percentages, ranging from 99.38 to 99.71, were discovered. The results are given in table 3 [33].

**Precision**

In method precision study, prepare six different standard solutions in the concentration of sotorasib (100 µg/ml) are injected into HPLC system. Sotorasib % assay was found to be in the range of 99.3-101.2. Peak areas were calculated, which were used to calculate mean, SD and %RSD values. These results are given below table 4.

Table 3: Results of accuracy

S. No.	% Level	Sotorasib % recovery*	Average % recovery
1	50	99.7	100.5
		101.3	
		100.5	
2	100	100.2	100.3
		100.0	
		100.7	
3	150	100.5	100.6
		100.5	
		101.0	

\*Mean+SD, n=3

Table 4: Intraday precision results of sotorasib

Sotorasib			
S. No.	Conc. (µg/ml)	Area counts	% assay as is
1	100	2632418	100.5
2		2601582	99.3
3		2614570	99.8
4		2628269	100.3
5		2617321	99.9
6		2651651	101.2
% RSD	0.66		
mean	100.2		
SD	0.656		

Mean+SD (n=6)

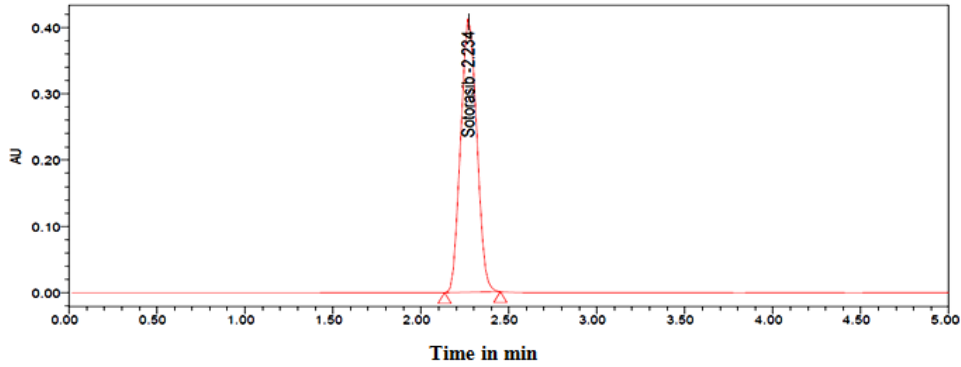


Fig. 5: Chromatogram of method precision

**Intermediate precision**

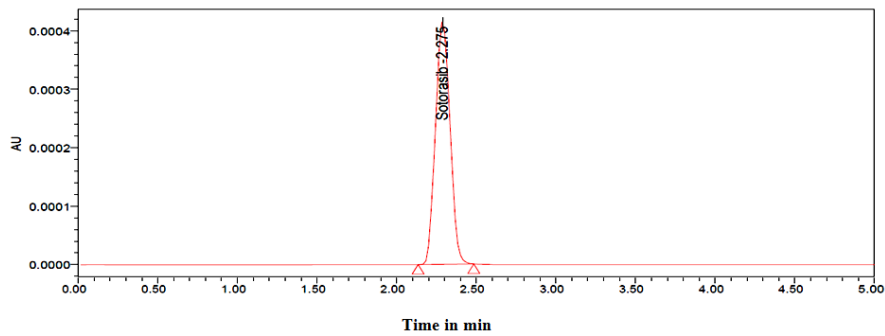
Separate instruments were used on different days, in different locations, for independent investigations into six different replicates of the standard solution. Mean RSD values have been calculated and determined from the peak regions. The following table shows the

results. Sotorasib (100µg/ml) was analysed on 6 different days with 6 different standards. Mean, standard deviation, and percent related standard deviation values were calculated from peak areas. Thus, it has been found that the current method yields very accurate results, with RSD values less than 2 percent and percent assay values near 100 percent. In table 5 [34] we can see the results.

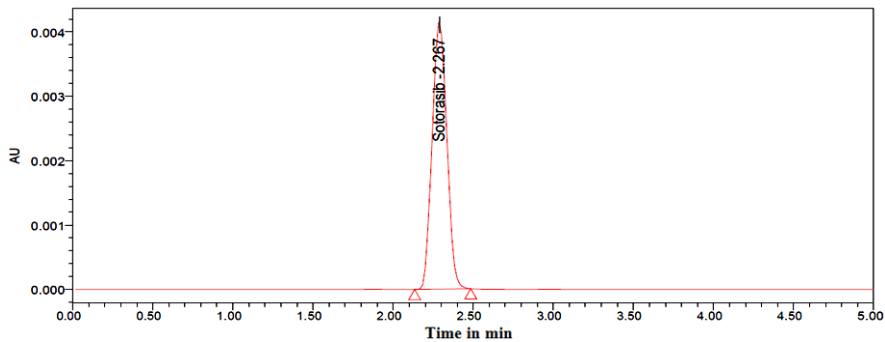
Table 5: Inter-day outcomes of the accuracy of sotorasib

Sotorasib			
S. No.	Conc. (µg/ml)	Area counts	% assay as is
1	100	2658214	101.5
2		2613206	99.7
3		2615427	99.8
4		2626539	100.2
5		2604362	99.4
6		2641488	100.8
%RSD	0.765		
Mean	100.23		
SD	0.787		

n=6



A



B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

**LOD and LOQ**

The LOD concentration sotorasib was 0.125 µg/ml and s/n values is 6. The LOQ concentration for sotorasib was 0.413 µg/ml and their s/n values are 25. The method is validated as per the ICH guidelines [35].

**Robustness**

The design of the experiment was intentionally altered in order to test the robustness of the system. Examples of such changes include changing the flow rate, organic to inorganic ratio, and so on. The results were robust and tabulated in table 7 [36].

**Table 6: LOD and LOQ for sotorasib**

Sotorasib			
LOD		LOQ	
Concentration	s/n	Concentration	s/n
0.125µg/ml	6	0.413µg/ml	25

**Stability**

The solution was kept at room temperature and between 2 and 8 degrees Celsius for up to 24 h [37]. The experiment showed that the

**Table 8: Stability results of sotorasib**

Stability	Stability at RT		Stability at 2-8 °C	
	% assay	% of deviation	% assay	% of deviation
Initial	100	0.00	100	0.00
6 h	99.6	-0.40	99.5	-0.50
12 h	99.3	-0.70	99.2	-0.80
18 h	98.7	-1.30	96.2	-3.80
24 h	96	-4.00	95.5	-4.50

**Acid degradation**

Acid degradation was done by using 1N HCl and 17.5% of Sotorasib degradation was observed.

**Alkali degradation**

Alkali degradation was done at 1N NaOH and 17.8% of Sotorasib degradation was observed.

**Peroxide degradation**

Peroxide degradation was performed with 30% hydrogen peroxide and 18.6% Sotorasib degradation was observed.

**Reduction degradation**

Reduction degradation was performed with 30% sodium bi sulphate solution, 16.9% sotorasib degradation was observed.

**Thermal degradation**

In thermal degradation, the standard was degraded to 13.9% of Sotorasib.

All degradation results are tabulated in table 9.

**Table 9: Forced degradation results of sotorasib**

Degradation condition	Sotorasib	
	% assay	%Deg
Acid degradation	82.5	17.5
Alkali degradation	82.2	17.8
Peroxide degradation	81.4	18.6
Reduction degradation	83.1	16.9
Thermal degradation	86.1	13.9

**CONCLUSION**

The developed method is accurate, precise and reliable for the analysis of Sotorasib in pharmaceutical formulations. This method

solutions remained stable for at least two days under room temperature and 2-8 °C, Sotorasib drug has no effect. The following table 8 illustrates the results.

**Table 7: Robustness data of sotorasib**

Parameter name	% RSD
Sotorasib	
Flow minus (0.8 ml/min)	0.63
Flow plus (1.2 ml/min)	0.51
Organic minus (63:37)	1.29
Organic plus (77:23)	0.88

**Degradation studies**

Partial degradation of the drug was accomplished using various forced degradation conditions on the Sotorasib standard. Research has been carried out to see if the method works for degrading products [38, 39]. Additionally, the studies describe the conditions under which the drug is unstable, providing further information so that appropriate precautions are taken during the process of formulation in order to avoid possible instabilities [40].

was validated for linearity, accuracy, precision, robustness, forced degradation and stability of Sotorasib. The RSD values for all parameters were found to be less 2, which indicates the validity of method and results obtained by this method are in fair agreement. Finally, this method can be used for better analysis of Sotorasib.

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**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICT OF INTERESTS**

Declared none

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