CHIRAL HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF S-SOTORASIB AND FD CHARACTERIZATION BY MS

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ABSTRACT:

The goal of the current study was to create and validate a novel CHIRAL-HPLC approach for estimation of S-Sotorasib in presence of its known impurity1and FD characterization by MS. To select the optimal chromatographic conditions, many trails were run; the best trail was selected for the optimized process. Using a PDA detector-equipped with high performance liquid chromatography. The wavelength of maximum absorption of the S-Sotorasib and its known impurity1 in the mixture of isopropyl alcohol and n-hexane (20:80v/v, mobile phase) was showed at 287 nm (Isobestic point) and exhibits linearity in the concentration range 30-180µg/ml for S-Sotorasib and 2.5-15µg/ml for impurity1. The Column used was Chiralcel OD-H150X4.6mm, 5µ. As part of method validation, the suggested method was determined in compliance with the analytical parameters listed in the ICH guidelines, such as system suitability, accuracy, precision, specificity, linearity, robustness, LOD, and LOQ. The results were found to be within the acceptable range. The drug subjected to different forced degradation studies such as Acid hydrolysis, Alkali hydrolysis, oxidation, and Reduction, Thermal and Photolytic degradation. The obtained total four degradative products were subjected to Mass characterization to elucidate the S-Sotorasib impurity profiling. The current study concluded that the developed method was simple, economical, accurate, precise, stable, and effective. It doesn't interfere with degradative products and placebo. Therefore, analysis of S-Sotorasib in the presence of impurities can be done using this method.

KEYWORDS:

S-Sotorasib, CHIRAL-HPLC, MS, Degradative products

INTRODUCTION:

IUPAC Name: 6-Fluoro-7-(2-fluoro-6-hydroxyphenyl) - (1*M*)-1-[4-methyl-2-(propan-2- yl) pyridin-3- yl]-4-[(2*S*)-2- methyl-4-(prop-2-enoyl) piperazin-1-yl] pyrido [2, 3-*d*] pyrimidin-2(1*H*)-one

Molecular Formula $C_{30}H_{30}F_2N_6O_3$ [1]

Molecular Weight $560.606 g \cdot mol^{-1}$

Non-small cell lung cancer (NSCLC) can be treated with both locally advanced (cannot be removed by surgery) and metastatic (cancer that has already spread) types. [2] Sotorasib binds to the cysteine residue KRASG12C to lock the protein in its inactive form, which inhibits cell proliferation and promotes apoptosis [3]. Because wild-type KRAS lacks this residue, off-target effects are reduced. According to a review of the literature, some analytical techniques were created utilizing LC-MS [6] and HPLC [4-5]. There was no technique created to separate Sotorasib enantiomers using chiral columns in presence of known impurities. Hence, the authors have created a chiral HPLC approach that has been verified for S-Sotorasib quantification and impurity profiling. Structure of S-Sotorasib and Impurity-1 were illustrated in figure 1 and figure 2.

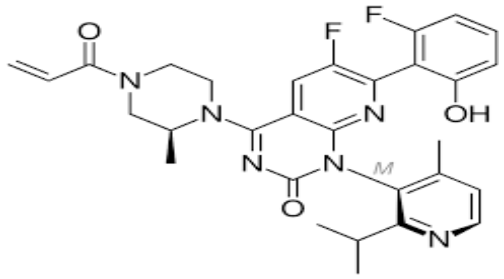


Figure 1: Structure of S-Sotorasib

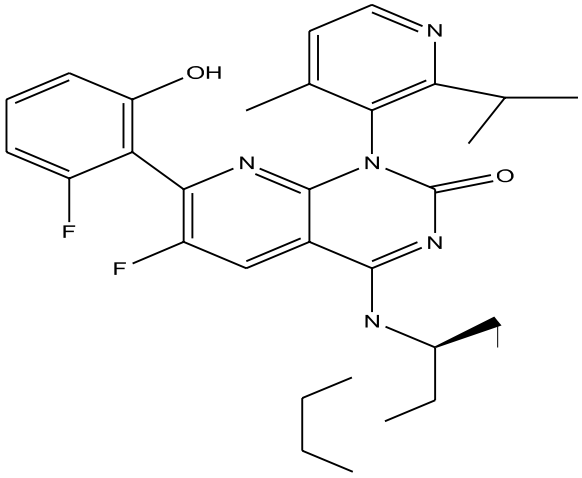


Figure 2: structure of Impurity

MATERIAL AND METHODS:

Method Development

A) Instruments

For analysis, the Waters Alliance e-2695 model HPLC with column oven, auto sampler, and degasser was turned on. The SCIEX QTRAP 5500 mass spectrometer, which has an electrospray ionization interface, was connected to the HPLC system. The chromatographic data was interpreted using the SCIEX program. pH meter PICO+- LAB INDIA, measuring device: Eutech Balance-Sartorius, Shimadzu UV-1800 spectrophotometer and Unichrome ultrasonicator

B) Reagents & Chemicals

Isopropyl Alcohol – HPLC grade

N-hexane _HPLC grade

Water _ HPLC Grade

Drug Samples:

SOTORASIB(S/R), S-SOTORASIB its related impurity -1 were obtained as a gift sample from Shree icon labs, Vijayawada, India.

Preparation of Racemic Mixture Standard Solution (stock-A):

Precisely measure and transfer 120 mg of Sotorasib working standard into a 10 ml dry volumetric flask. Then, add diluents, sonicate to dissolve the Sotorasib fully, and use the same solvent (stock solution) to get the volume up to the required level. Pour 1 milliliter of the aforementioned solution into a 10-milliliter volumetric flask and add diluents to reach the desired level.

Preparation of S-isomer standard stock solution (stock-B): 120 mg of Sotorasib (S-isomer) working standard should be properly weighed and transferred into a 10 ml dry, clean volumetric flask. Subsequently, incorporate the diluents, sonicate the sample until it dissolves completely, and utilize the same solvent to raise the volume to the necessary level (stock solution-B).

Preparation of Impurity-1 Stock Solution (stock-C): Accurately weigh out 10 mg of Sotorasib impurity-1, and then transfer it into a 10 ml volumetric flask. Add 5 ml of diluents, sonicate for 30 minutes, and then use the same diluents to bring the volume up to the required level. Pour 1 milliliter of the aforementioned solution into a 10-milliliter volumetric flask and add diluents to reach the desired level.

Preparation of Spiked standard solution (stock-D): Fill a 10-milliliter volumetric flask to the brim with diluents after adding one milliliter each of the standard stock solution (s-isomer) and the impurity stock solution. Use a 0.45µ membrane filter to filter the solution.

Preparation of Mobile Phase: The mobile phase was made by combining n-hexane at a 20:80 ratio with isopropyl alcohol. A 0.45µ membrane filter was used to filter it out of any impurities that would have affected the final chromatogram.

Determination of Working Wavelength (λ_{max}):

The method was utilized to estimate the drug's isobestic wavelength. The isobestic point refers to the wavelength at which the molar absorptivity of inter convertible substances is identical. Hence, this wavelength allowed for a precise assessment of the drug. Using a PDA detector, the wavelength corresponding to the maximum absorption of the drug and impurity-1 in a mixture of isopropyl alcohol and n-hexane (20:80) were analyzed against the same mixture serving as a blank, within the 200–400 nm wavelength range. The absorption curve indicated the isobestic point at 287 nm. Therefore, 287 nm was chosen as the detection wavelength for the HPLC method.

Chromatographic condition:

Column	: Chiralcel ODH 150X4.6mm, 5µ.
Mobile phase	: Isopropyl alcohol and n-hexane (20:80 v/v)
Detection Wavelength	: 287nm
Flow rate	: 1.5ml/min
Injection volume	:10µl
Column oven temperature	:Ambient temperature
Runtime	:15min

METHOD VALIDATION

SYSTEM SUITABILITY:
It is important to perform a system appropriateness test to ensure that the analytical system is operating correctly and producing exact results. Following the test protocol, a working standard solution of S-Sotorasib and its impurity-1 was made, and it was injected six times into the HPLC apparatus. S-Sotorasib and its impurity-1 in standard solution should have a tailing factor of not more than 2.0.

SPECIFICITY:

The capacity of an analytical method to detect an analyte of interest precisely without interference from known and blank impurities is known as specificity. Three chromatograms— blank, standard, and sample were recorded for this purpose**.**

LINEARITY:

Using the appropriate dilutions with mobile phase, a series of aliquots were made, ranging from 30 ug/ml to 180 ug/ml for S-Sotorasib and 2.5 to 15 ug/ml for imp-1. Measure the peak area after injecting each concentration into the chromatographic apparatus six times. Plot the average peak area against the concentration on a graph, and then calculate the correlation coefficient.

ACCURACY:

Study of accuracy for S-Sotorasib and its Imp-1 was conducted in triplicate (50%, 100%, and 150%) using the same amount of drug containing Sotorasib (S-isomer) and its Imp-1 into each volumetric flask for each spike level. A percentage recovery average was computed.

PRECISION:

A homogeneous sample from a single batch should be examined six times for precision of method. % RSD was calculated for peak area and retention times.
The instrument's accuracy was verified by repeatedly injecting (n=6) 120 ppm Sotorasib (S-isomer) solutions.

ROBUSTNESS:

Deliberate adjustments were made to the Flow rate and Mobile Phase composition as part of the Robustness to assess the effect on the procedure.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ):

As per ICH guidelines, the limits of detection (LOD) and quantification (LOQ) for the drug were established using the formula. The LOD is defined as 3.3 times σ/S. The LOQ is defined as 10 times σ/S.

FORCEDDEGRADATION STUDIES:

Acid Hydrolysis:

A 10 ml volumetric flask was filled with 1 ml of S-Sotorasib (stock-B) and 1 ml of 1N HCl. After being in a water bath at 60°C for one hour, the volumetric flask was neutralized using 1 N NaOH and brought up to a total volume of 10 ml with diluents. Transfer the solution into vials by using 0.45 micron filter. Inject the vial into HPLC for analysis. Chromatogram and purity plot of acid hydrolysis were illustrated in figure 9 and 10.

Alkali Hydrolysis:

Take 1ml of S-Sotorasib(stock-B) into a 10 ml volumetric flask, and 1 ml of 1N NaOH was incorporated. Following an hour at 60 degrees Celsius, the solution in the volumetric flask was neutralized with 1N HCl and adjusted to a final volume of 10 ml with diluents. Transfer the solution into vials by using 0.45 micron filter. Inject the vial into HPLC for analysis. Chromatogram and purity plot of alkali hydrolysis were illustrated in figure 11 and 12.

Oxidative degradation

10 ml volumetric flask was filled with 1 ml S-Sotorasib stock solution and 1 ml 30% H2O2, add diluents to increase the volume to desired level. The volumetric flask was then held at 60°C for one hour. After held at room temperature for 15 minutes. Transfer the solution into vials by using 0.45 micron filter. Inject the vial into HPLC for analysis. Chromatogram and purity plot of Peroxide degradation were illustrated in figure 13 and 14.

Reduction

10 ml volumetric flask was filled with 1 ml S-Sotorasib stock-B solution and 1 ml 10% sodium bisulphate, the volume was increased to the required level using diluents. The volumetric flask was then kept at 60 °C for one hour. After then allowed to stand at room temperature for 15 minutes. . Transfer the solution into vials by using 0.45 micron filter. Inject the vial into HPLC for analysis. Chromatogram and purity plot of Reduction degradation were illustrated in figure 15 and 16.

Photolytic degradation

For 6 hours, the wrapped control sample and the standard Sotorasib (S-isomer) were kept in the photo stability chamber. Subsequently, the material was extracted, diluted using diluents. . Transfer the solution into vials by using 0.45 micron filter. Inject the vial into HPLC for analysis. Chromatogram and purity plot of Photolytic degradation were illustrated in figure 17 and 18.

Thermal degradation

Sotorasib (S-isomer) working standard was placed in a Petridish and heated to 105°C in an oven for three hours. The sample was then diluted with diluents and placed in an HPLC for analysis. Chromatogram and purity plot of Thermal degradation were illustrated in figure 19 and 20.

Mass spectrometer conditions: -

The interface mode of the mass spectrometer was set on the positive ionization, MRM was used for analysis. Temperature: 550 ° C; Drying temperature: 120-250 ° C; Collision energy: 15V; Ion spray voltage: 5500 V; and collision gas: nitrogen.5 ml/min of dry gas flow; 40 V clustering potential; 10V admission potential; 7 V exit potential

RESULTS AND DISCUSSION:

Using a Chiralcel OD-H column (150 mm x 4.6 mm, 5 µm), the developed chiral HPLC method allows the simultaneous measurement of S-Sotorasib and its impurities. The mobile phase consists of isopropyl alcohol and n-hexane in a ratio of 20:80 v/v at a flow rate of 1.5 ml/min. The column was maintained at ambient temperature while detection was performed at 287 nm.

Optimized condition for S-Sotorasib & impurity:

Retention time of S-Sotorasib and Sotorasib Imp-1 were about 2.509 and 4.732min. Chromatogram of S-Sotorasib and impurity-1 were illustrated in figure-3.

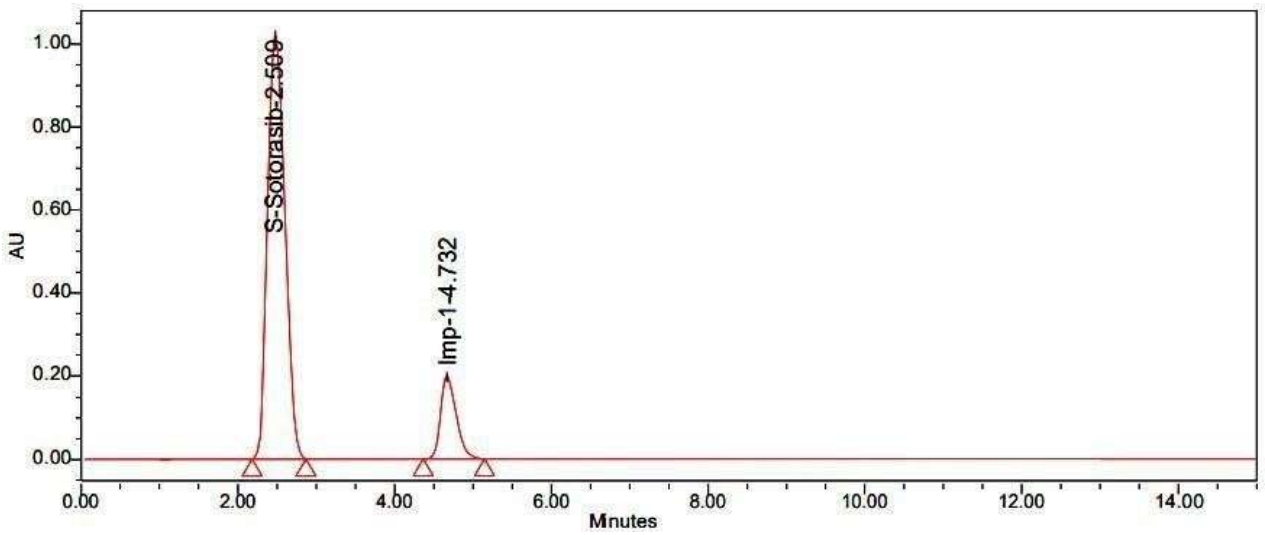


Figure 3: Chromatogram of S-Sotorasib & impurity

System suitability:

The system suitability was performed by injecting spiked solutions into the HPLC system six times. It was observed that all parameters were within the limits. The results were shown in Table 1.

Table 1: Results of System Suitability

Parameter	s-Sotorasib (n=6)	Imp-1 (n=6)
Retention Time	2.506min	4.731min
Theoretical Plates	16889	12064
Tailing Factor	0.96	0.84
USP Resolution	-	4.51
%RSD of peak areas	0.74	0.45

Linearity and Range

For Sotorasib (S-isomer) and Imp-1, the linearity approach was proven over concentration range of 30-180 µg/ml and 2.5-15 µg/ml, respectively. The outcomes were displayed in Table 2. Calibration curve of S-Sotorasib and Imp-1 were illustrated in figure 4 and 5.

Figure 4: Calibration curve of S-Sotorasib

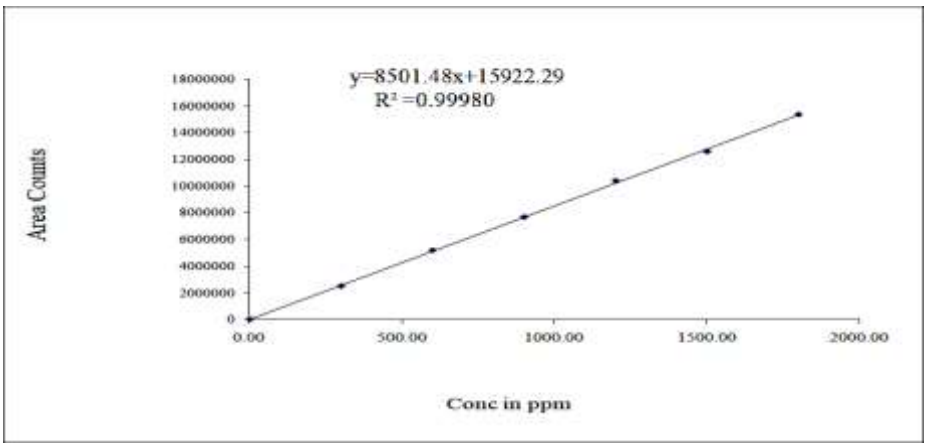


Figure 5: Calibration curve of Imp-1

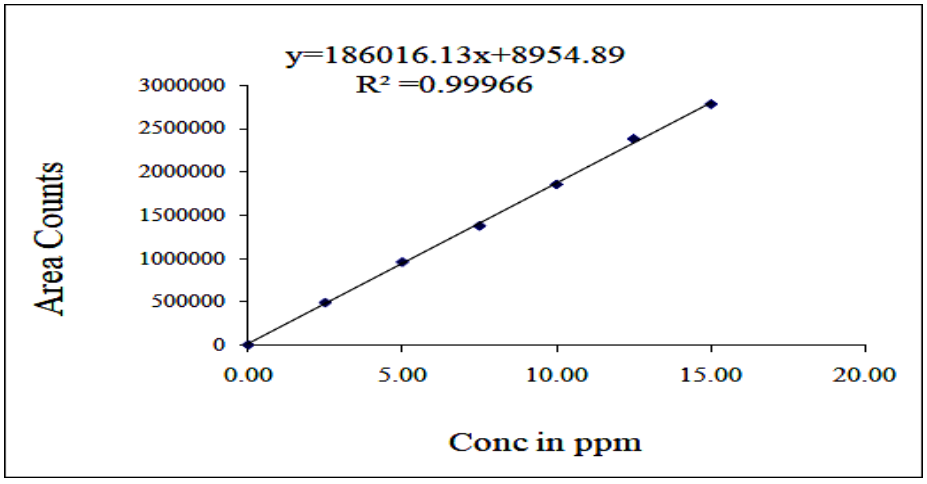


Table-2 linearity Parameters for S-Sotorasib and Imp-1

Parameters	Sotorasib	Imp-1
Linearity range	30-180µg/ml	2.5-15 µg/ml
Correlation coefficient	0.99980	0.99966

Precision:

a. System Precision (Repeatability)

Six injections were performed from the same standard preparations for injection repeatability, and the relative standard deviation for replicate injections was calculated. Table 3 showed the precision readings of the system.

Table-3 System Precision values for Sotorasib and Imp-1

DRUG	MEAN(n=6)	STDdev.	%RSD
S-SOTOROSIB	10373540	76734.056	0.74
Imp-1	1846001	8267.024	0.45

The system precision studies have shown that all parameters, such as %RSD of peak areas, are within limits.

b. Method Precision (intraday)

Six preparations of distinct samples from the same homogeneous mixture of marked sample were used to make the precision of the method. After saving the chromatograms, the average, the standard deviation and the %RSD were calculated. Table 4 displayed the results.

Table 4 Method precision values for S-Sotorasib and Imp-1

DRUG	MEAN (n=6)	SD	%RSD
S-SOTOROSIB	10425370	106668.745	1.02
Imp-1	1838692	11019.697	0.60

All of the parameters, including the peak area and retention time's %RSD, were found to be within the limits by the Method precision investigations.

Accuracy:

Recovery studies were conducted using standard addition method and table 5 shows the obtained results and those were good agreement with the labeled claim.

Table: 5 Accuracy Results for S-Sotorasib and Imp-1

RECOVERY LEVEL	TARGET CONC		AMOUNT TAKEN	%MEAN RECOVERY(n=6)	%RSD
	S-SOTORASIB	Imp-1			
50%	60	10	30	99.4	0.29
100%	60	10	60	99.9	0.84
150%	60	10	10.6	100.6	1.23

SPECIFICITY:

A. S-Sotorasib and Imp-1 Identification

Following the test protocol, solutions of the standard and the sample were made and injected into the system. Chromatogram of standard and sample were illustrated in figure 6 and 7.

Figure 6: Chromatogram of S-Sotorasib and Imp-1 (Standard)

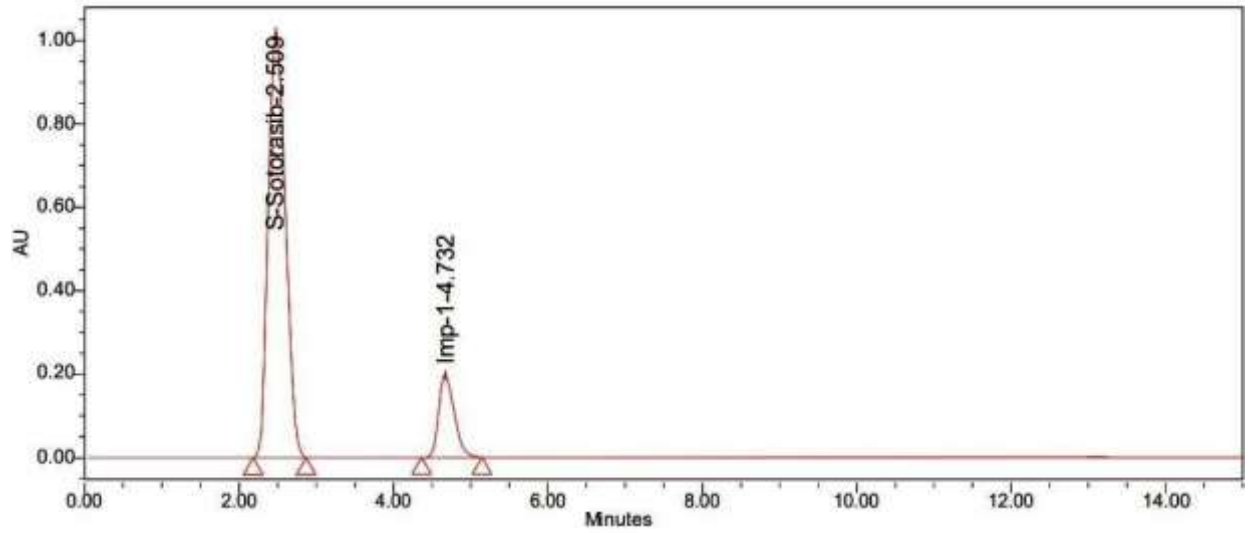
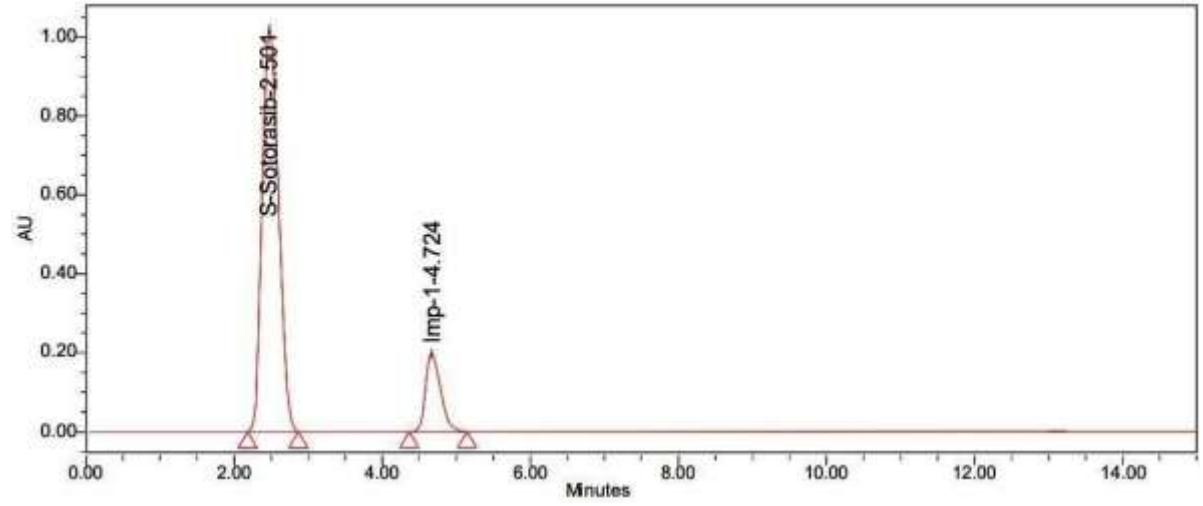


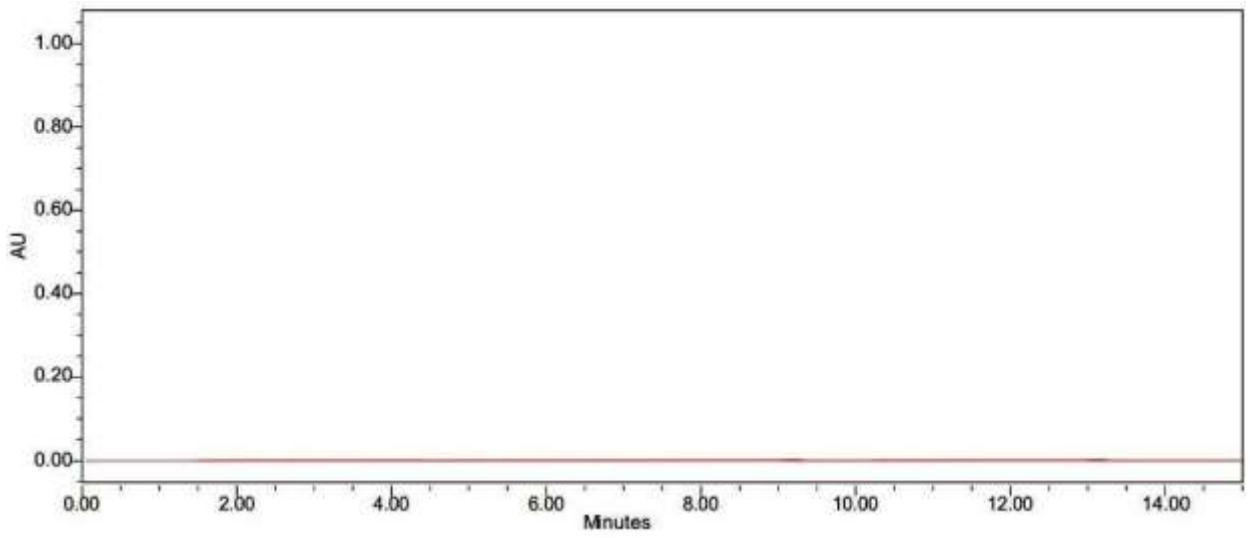
Figure 7: Chromatogram of S-Sotorasib and Imp-1 (Sample)



A. Blank Interference

An investigation was carried out to determine the interference of blank. As instructed by the test protocol, diluent was introduced into the HPLC apparatus. The obtained chromatograms revealed that no interference by the placebo and diluents, hence the method was specific. Figure 8 shows the chromatogram of diluents.

Figure 8: Chromatogram of Diluents:



Robustness

a. Effect of flow rate:

After preparing a test solution and injected into the HPLC at flow rates of 1.65 ml/min and 1.35 ml/min (±0.15 ml/min), the impact was assessed. The table 6 included the observations of flow rate.

Table 6 Robustness data-flow rate of mobile phase

DRUG	FLOW RATE	PEAK AREA	RT in min	%RSD
S-SOTOROSIB	1.35	9916243	2.711	0.12
	1.65	12645782	2.436	0.76
IMP-1	1.35	1672653	4.832	1.11
	1.65	1926451	4.585	1.00

Effect of Organic phase ratio:

A test solution was prepared and injected into the HPLC by making minute changes in the mobile phase composition. The results were shown in Table 7.

Table 7 Robustness data-organic phase in mobile phase

DRUG	COMPOSITION	PEAK AREA	RT in min	%RSD
S-SOTOROSIB	More Organic Phase	13126985	2.293	**0.87**
	Less Organic phase	9756487	2.853	**0.12**
IMP-1	More Organic Phase	2147653	4.471	**0.43**
	Less Organic phase	1524370	5.067	**0.93**

Robustness studies revealed that the method was unaffected by the minute changes.

Results of LOD and LOQ:

Table: 8 Limit of detection and limit of quantification

COMPOUND	µg/ml (n=6)	%RSD
SOTOROSIB	**LOD= 0.3**	0.43
	LOQ=1.2	0.52
IMP-1	**LOD=0.03**	0.34
	LOQ=0.1	0.41

LOD and LOQ results were mentioned in table 8. The results revealed that the method was set to be sensitive.

FORCED DEGRADATIONSTUDIES:

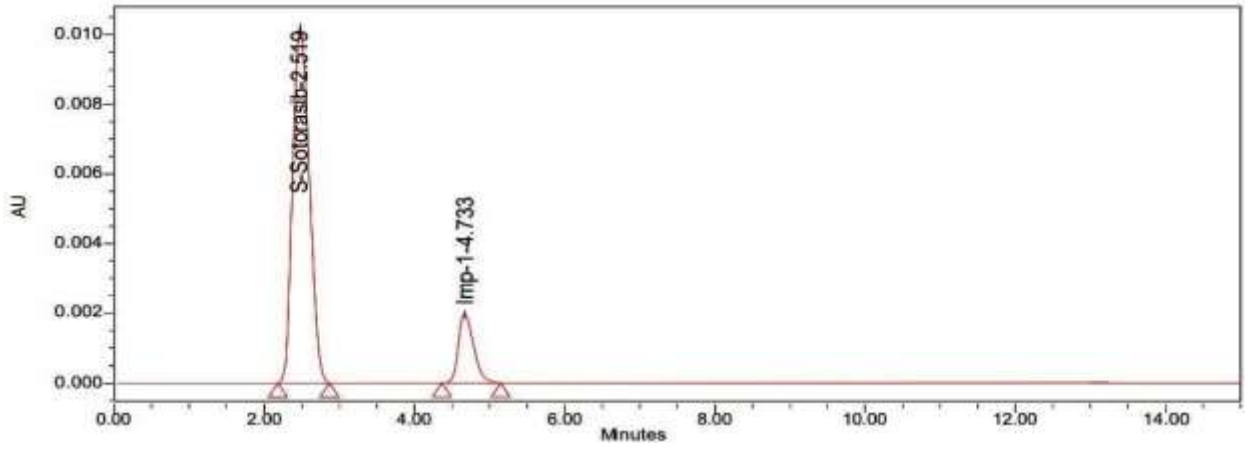


Figure 9: chromatogram for Acid Hydrolysis

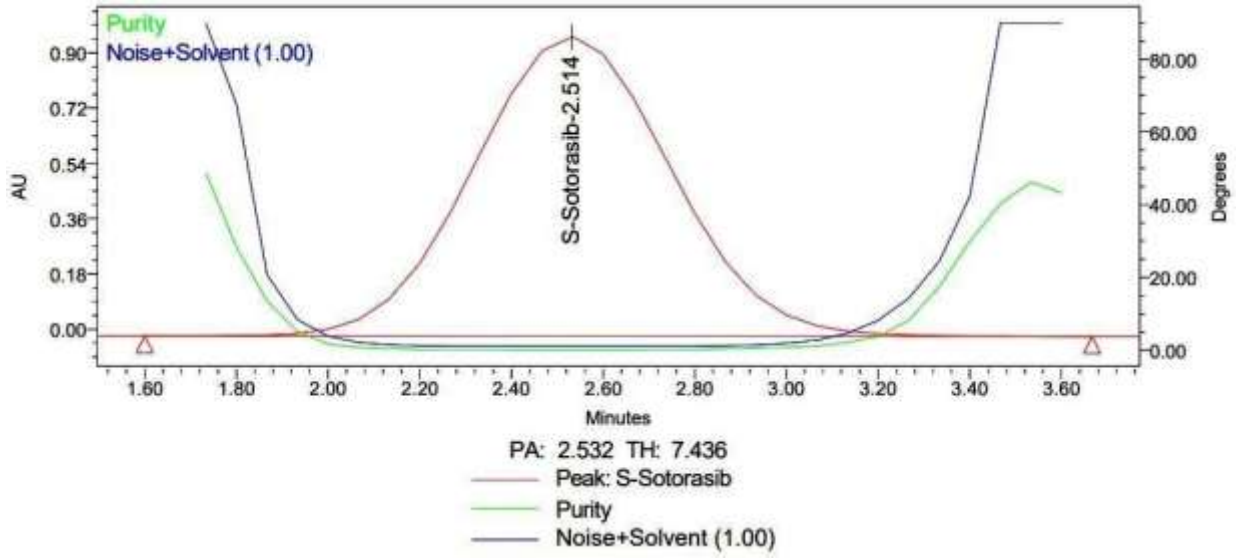


Figure 10: Purity plot of Acid Hydrolysis

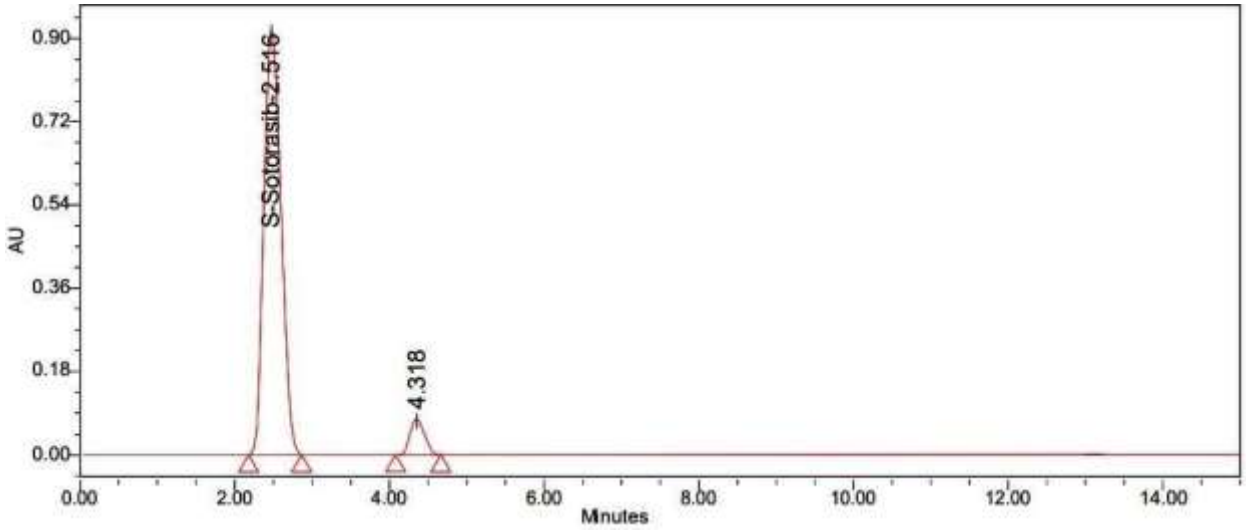


Figure 11: chromatogram for Alkali Hydrolysis

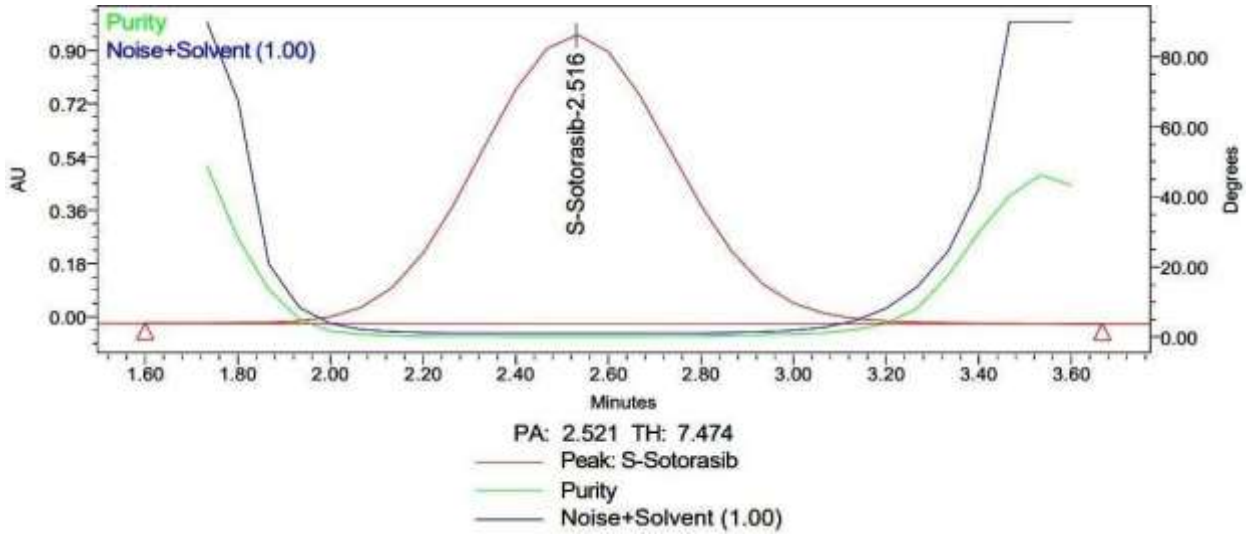


Figure 12: Purity plot of Alkali Hydrolysis

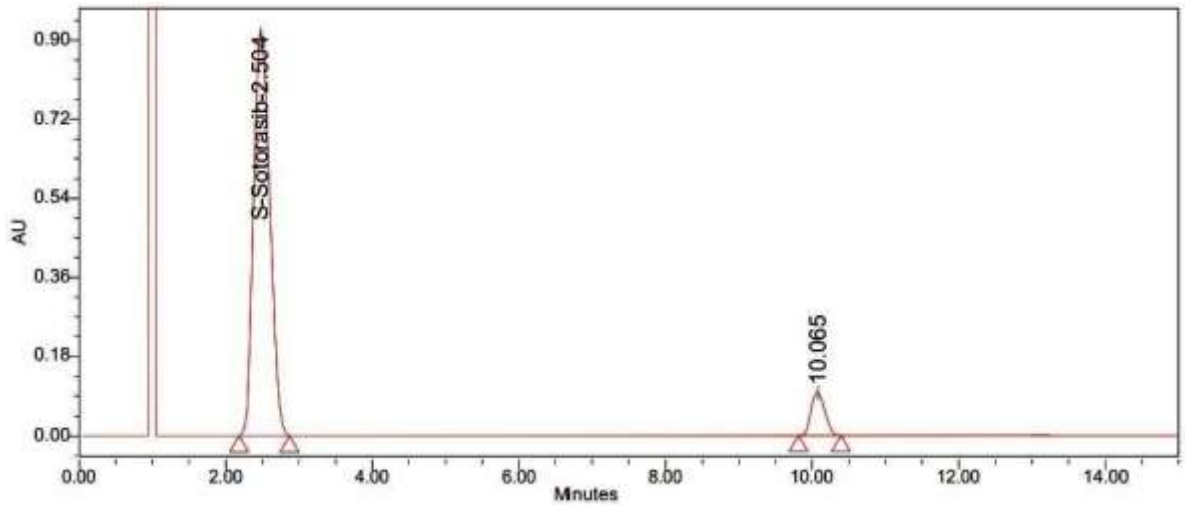


Figure 13: chromatogram for Peroxide degradation

Figure 14: Purity plot of Peroxide deg

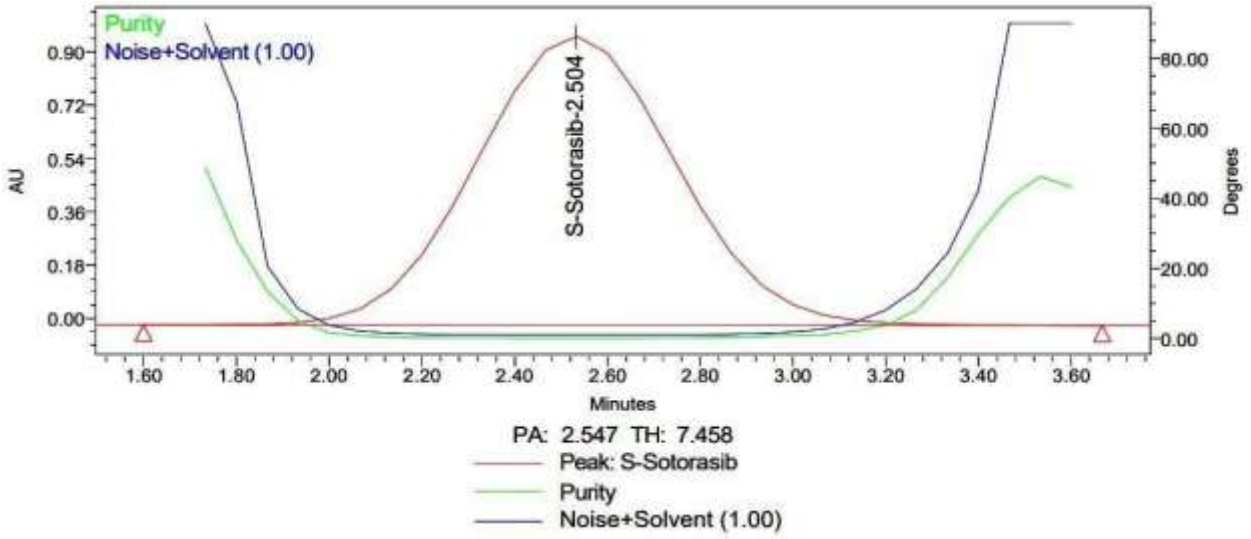


Figure 15: chromatogram for Reduction degradation

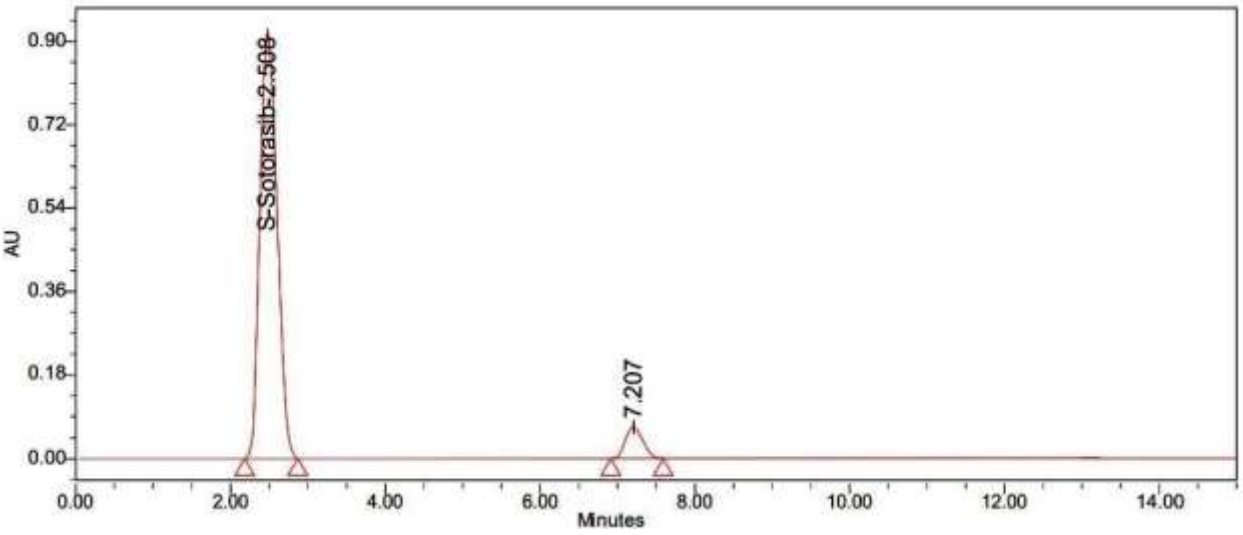


Figure 16: Purity plot of Reduction deg

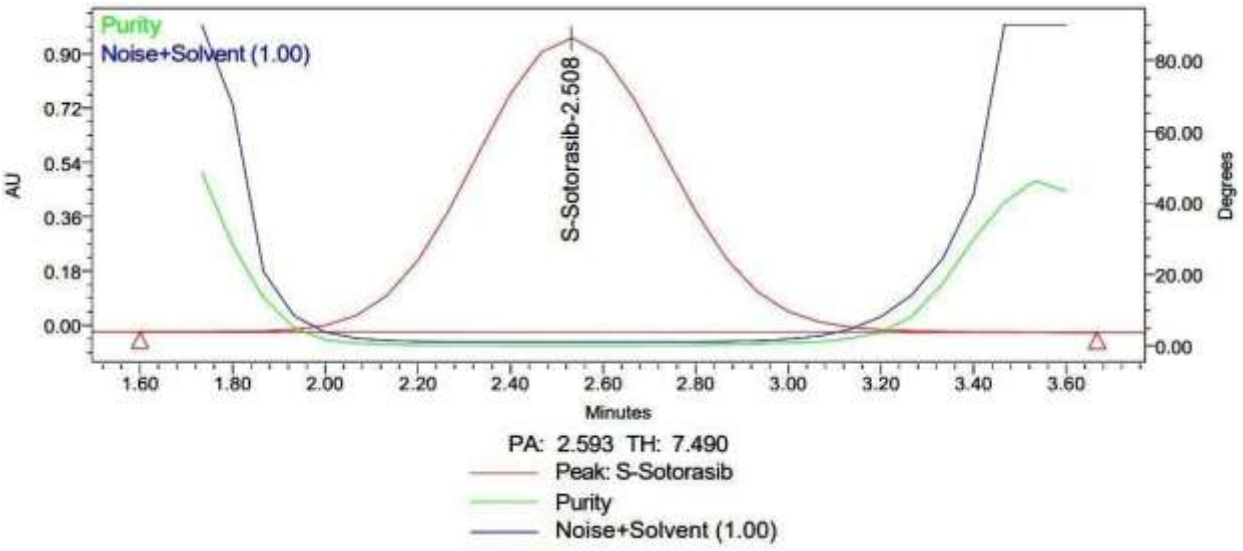


Figure 17: chromatogram for Photolytic degradation

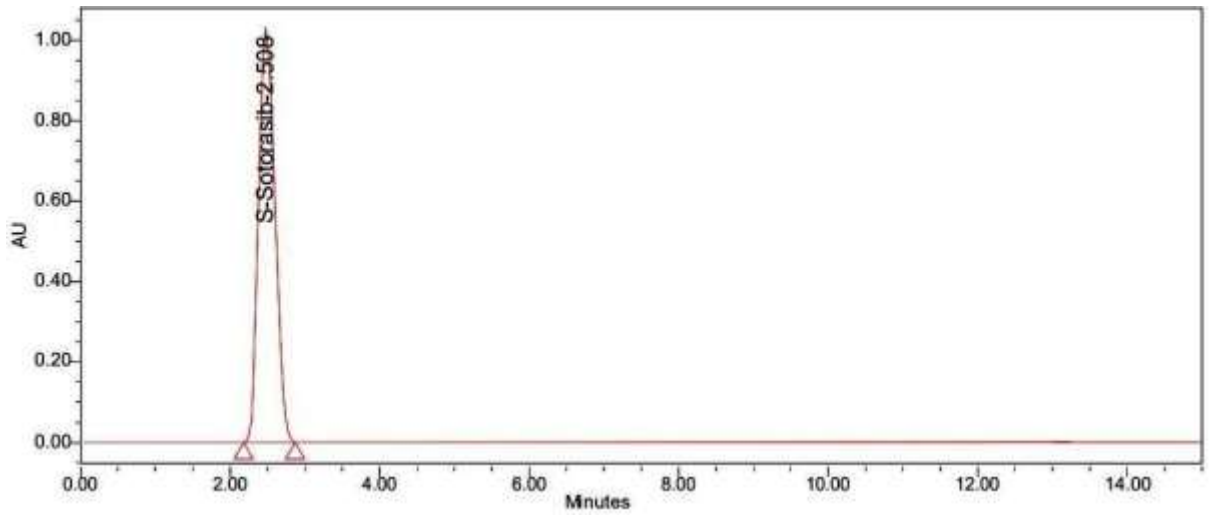


Figure 18: Purity plot of Photolytic

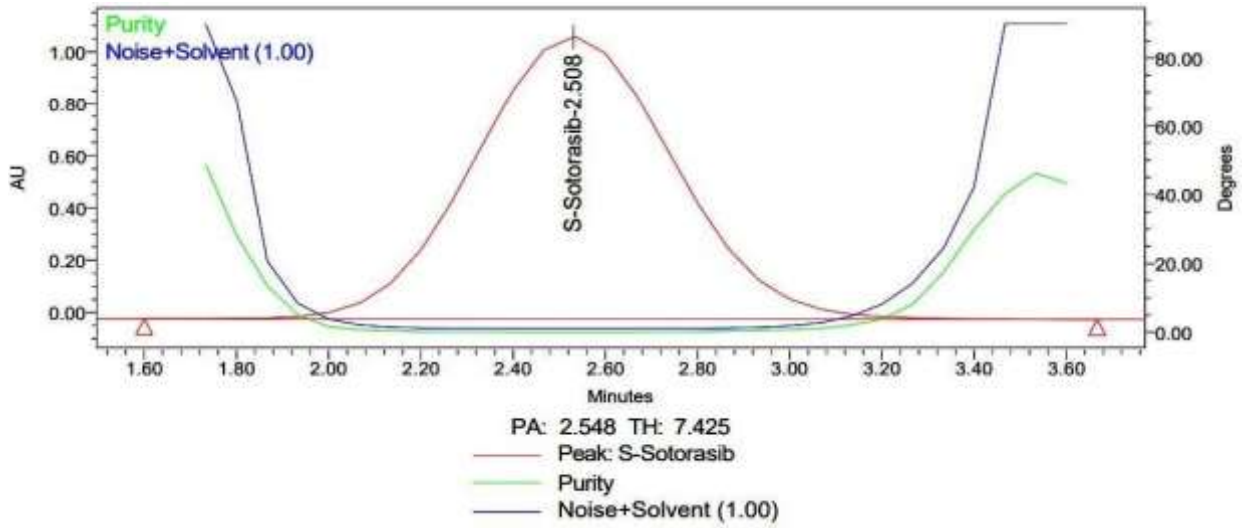


Figure 19: Chromatogram of Thermal degradation

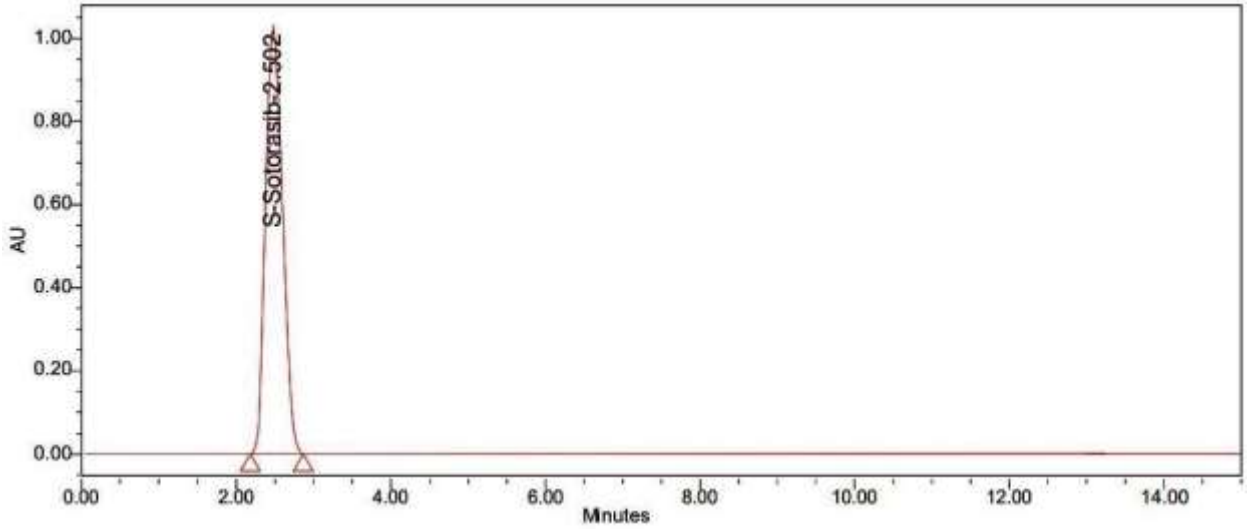


Figure 20: Purity plot of Thermal degradation

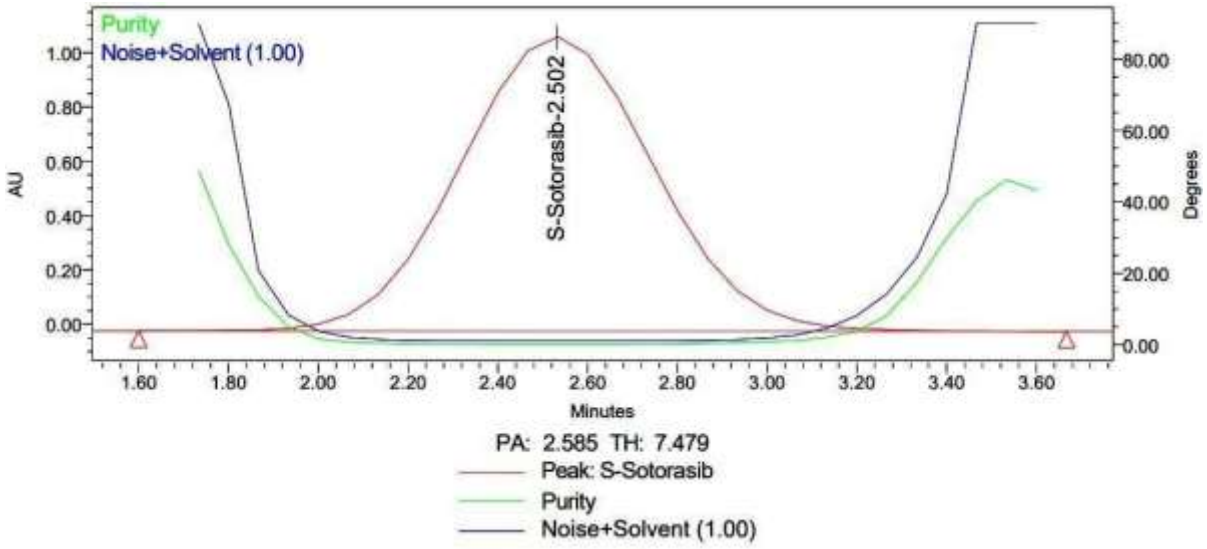


Table: 9 Forced Degradation results for S-Sotorasib

Stress conditions	% Assay after degradation	Purity Angle	Purity Threshold	%Degradation
Acid	88.8	2.532	7.436	11.2
Alkali	87.7	2.521	7.474	12.3
Peroxide	84.9	2.547	7.458	15.1
Reduction	87.6	2.593	7.49	12.4
Thermal	97.5	2.585	7.479	2.5
Photolytic	96.4	2.548	7.425	3.6

Table 9 shows the observed results of all the stressed conditions. In each forced degradation study, the purity angle is determined to be less than the threshold angle without purity flags.

Mass characterization of S-Sotorosib

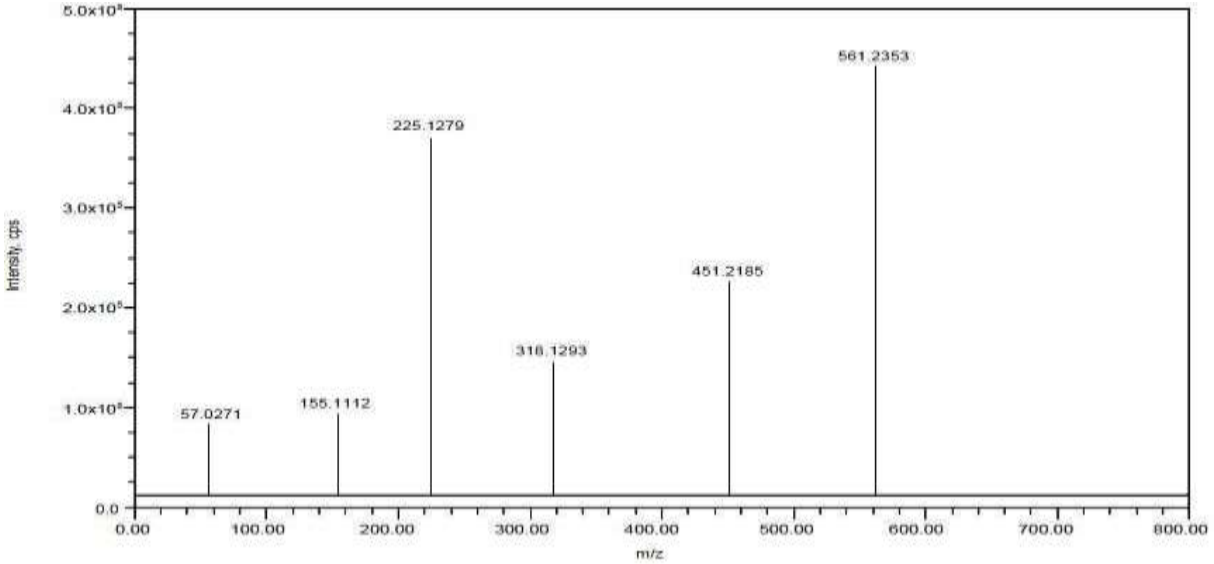


Figure 21 Mass spectra of s-sotorasib

Collision-induced dissociation of S-Sotorasib:

S-Sotorasib: The S-Sotorasib fragmentation pathway were illustrated in figure 22 and the ESI spectrum showed in figure 21, has been seen the ion [M+ H] + more intense than M/Z-560.2347. Abundant products have been observed in the MS/MS spectrum of S-Sotorasib in M/Z-450.2180 (loss of C6H5 of M/Z-560.2347), m/Z-317.1288 (loss of C9H13N from m/z 450.280), m/ Z 450.2180), M./Z-224.1273 (loss of C5H4FN of m/z 317.1288), m/z-154.1106 (loss of C2H4N2o of m/z 224.1273) and m/z-56.0262 (loss of C5H12N2 from m/Z 154.1106).The proposed fragmentation mechanism was validated by the MS/MS tests in combination with accurate mass measurements.

Figure 22: Fragmentation pathway of S-Sotorasib

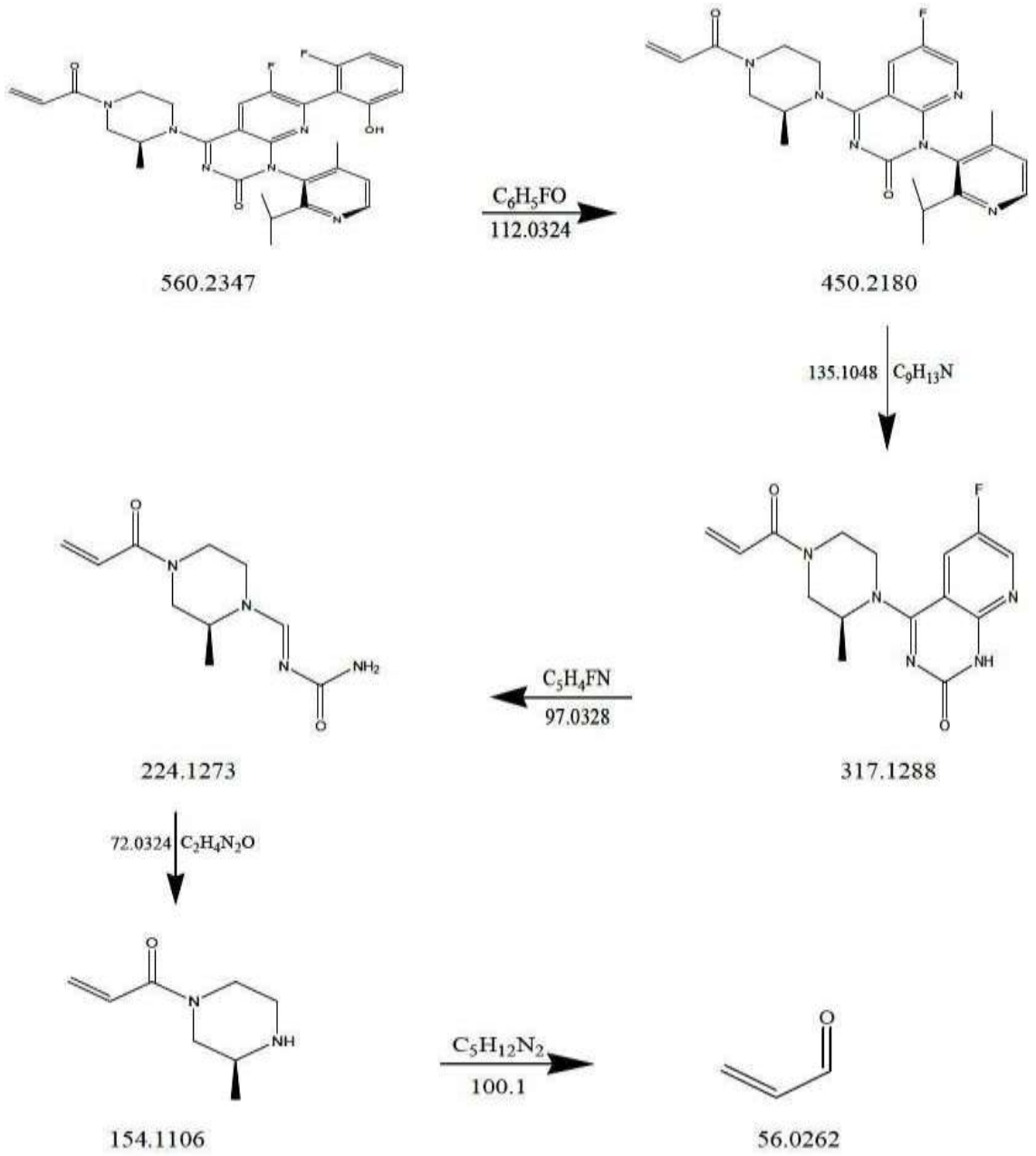
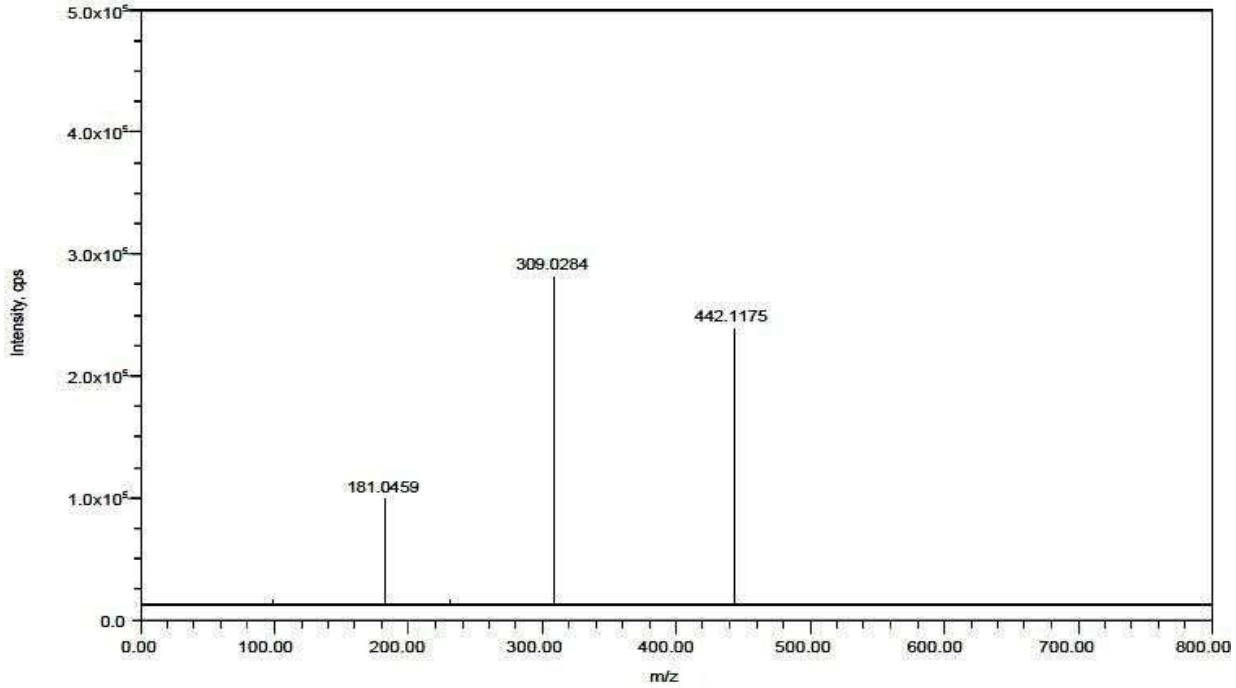


Figure 23: Mass Spectra of Acid Hydrolysis Degradation Product (DP-1)



DP1: The fragmentation pathway of DP1 was illustrated in figure 24, and the most intense [M+H] + ion of m/z-441.1168 was detected under conditions of acid degradation in the ESI spectrum. DP1's MS/MS spectra showed a lot of product ions at m/z-308.0276 (where C9H13N was lost from m/z-441.1168) and m/z-180.0447 (where C6H4ClF was lost from m/z 308.0276).

Figure 24: Fragmentation pathway of Acid Hydrolysis Degradation Product (DP-1)

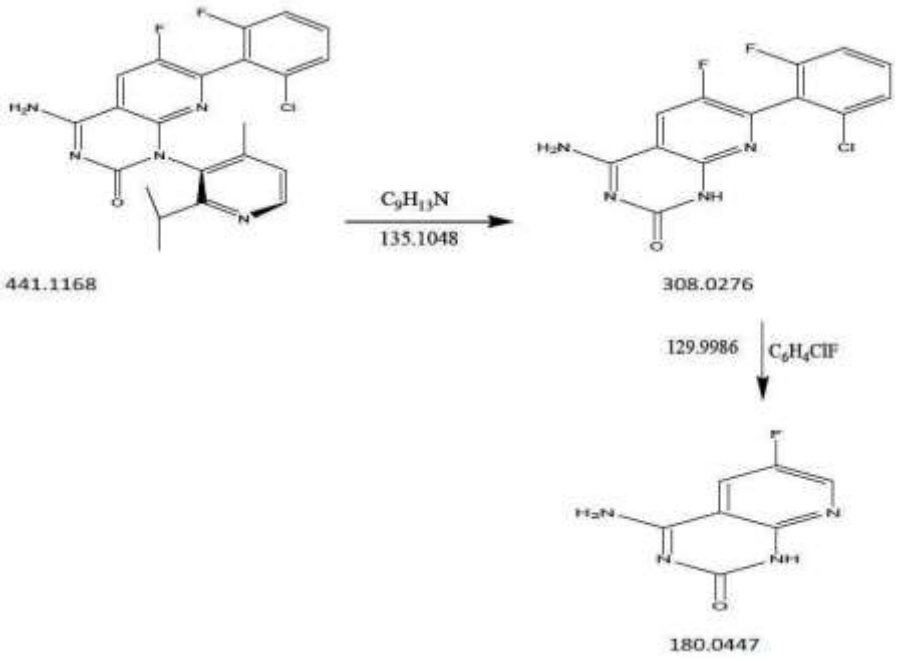
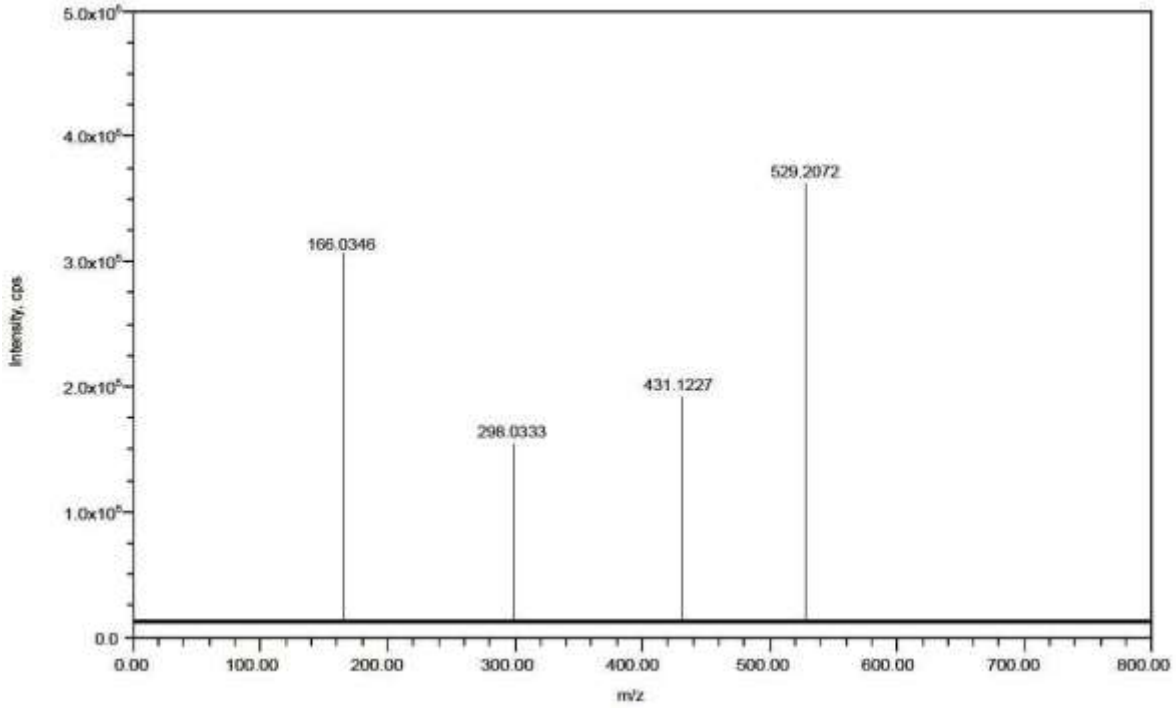
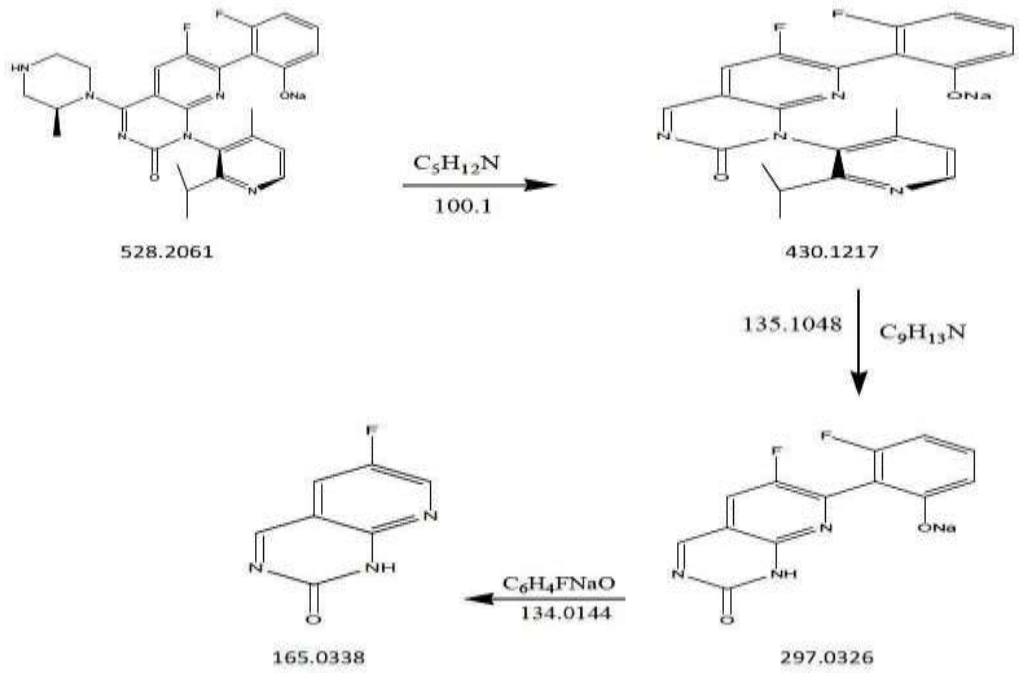


Figure 25: Mass Spectra of Alkali Hydrolysis Degradation Product (DP-2)

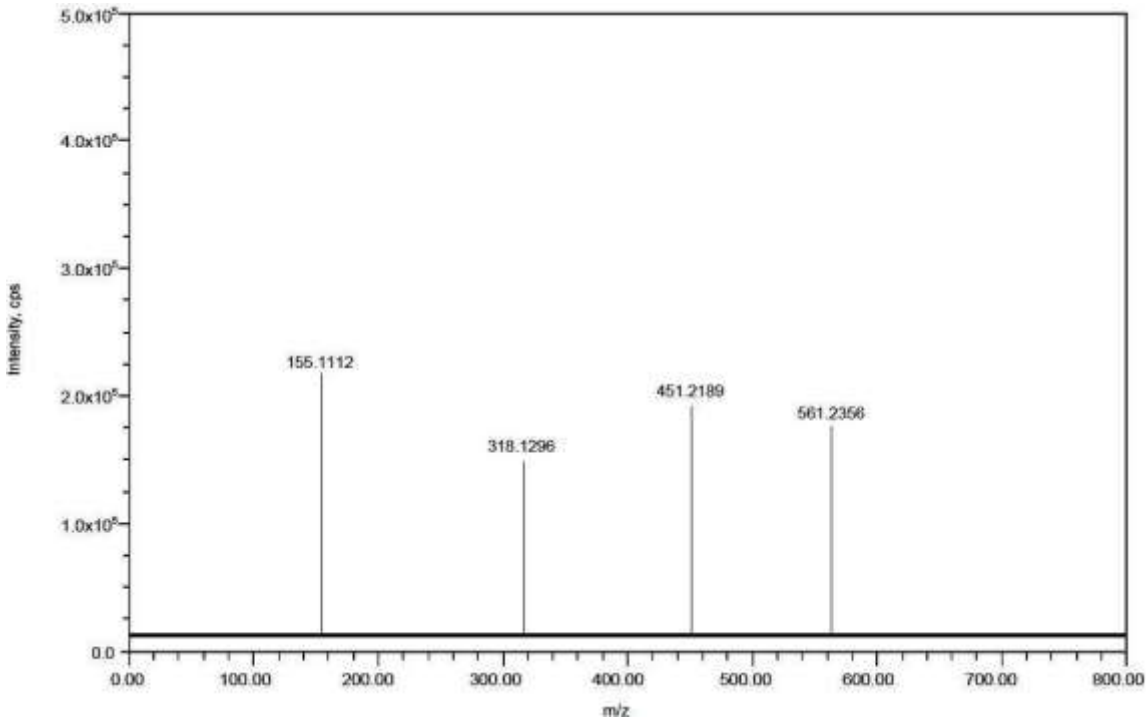


DP2: The fragmentation pathway of DP2 are illustrated in figure 26, and the most intense [M+H]+ ion of m/z-528.2061 was detected under conditions of alkali degradation, according to the ESI spectra. A lot of product ions were seen in the DP2 MS/MS spectrum at m/z-430.1217 (where C5H12N was lost from m/z-528.2061), m/z-297.0326 (where C9H13N was lost from m/z 430.1217), and m/z-165.0338 (where C6H4FNaO was lost from m/z 297.0326).

Figure 26: Fragmentation pathway of Alkali Hydrolysis Degradation Product (DP-2)

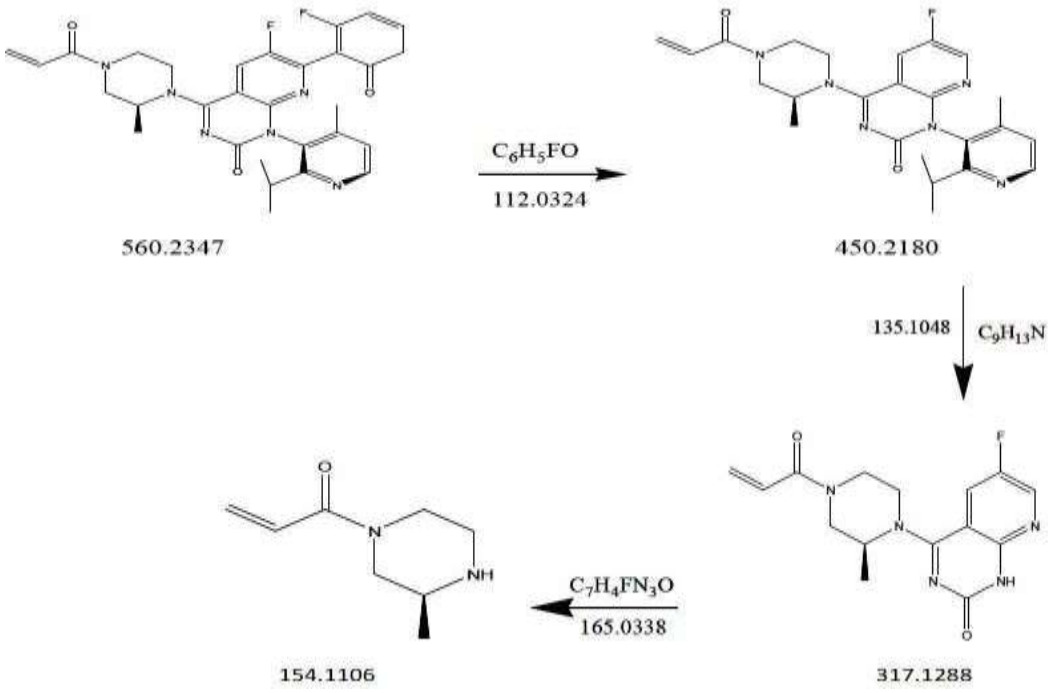


Figu r e 27: Mass Spectra of Peroxide Degradation Product (DP-3)

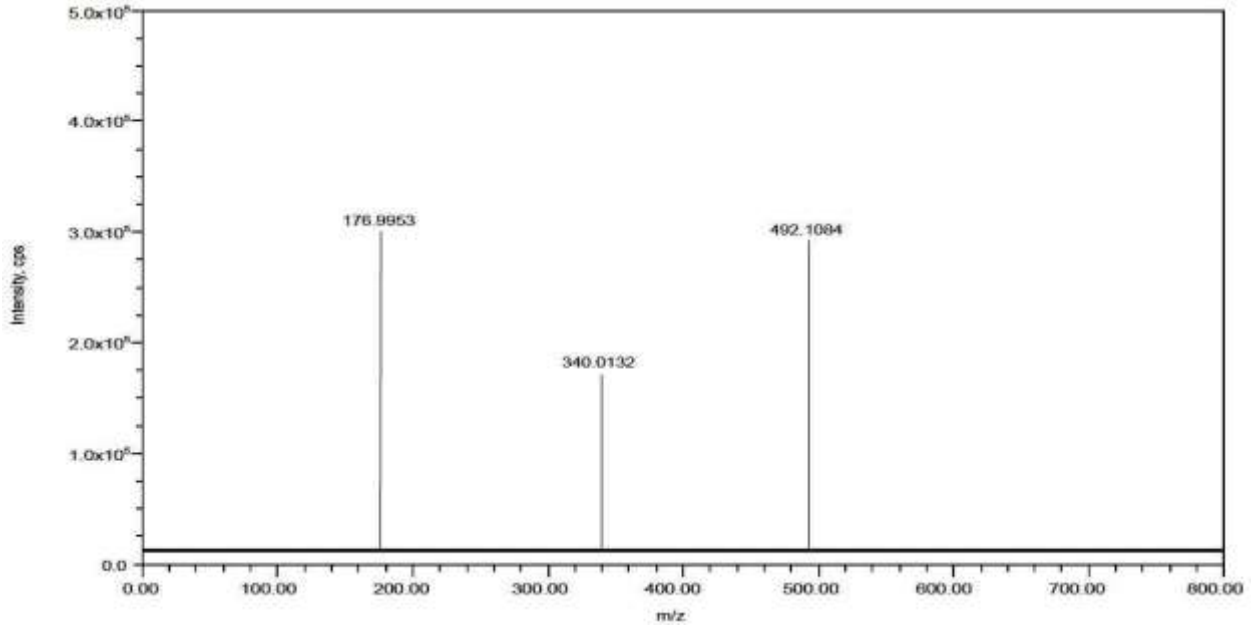


DP3: The ESI spectra revealed the most intense [M+H]+ ion of m/z-560.2347, which was found under Peroxide degradation conditions. The fragmentation mechanism of DP3. Abundant product ions were observed in the DP3 MS/MS spectra at m/z- 450.2180 (loss of C6H5FO from m/z-560.2347), m/z-317.1288 (loss of C9H13N from m/z 450.2180), and m/z-154.1106 (loss of C7H4FN3O from m/z 317.1288). The fragmentation pathways of DP-3 are illustrated in figure 28.

Figure 28: Fragmentation pathway of Peroxide Degradation Product (DP-3)

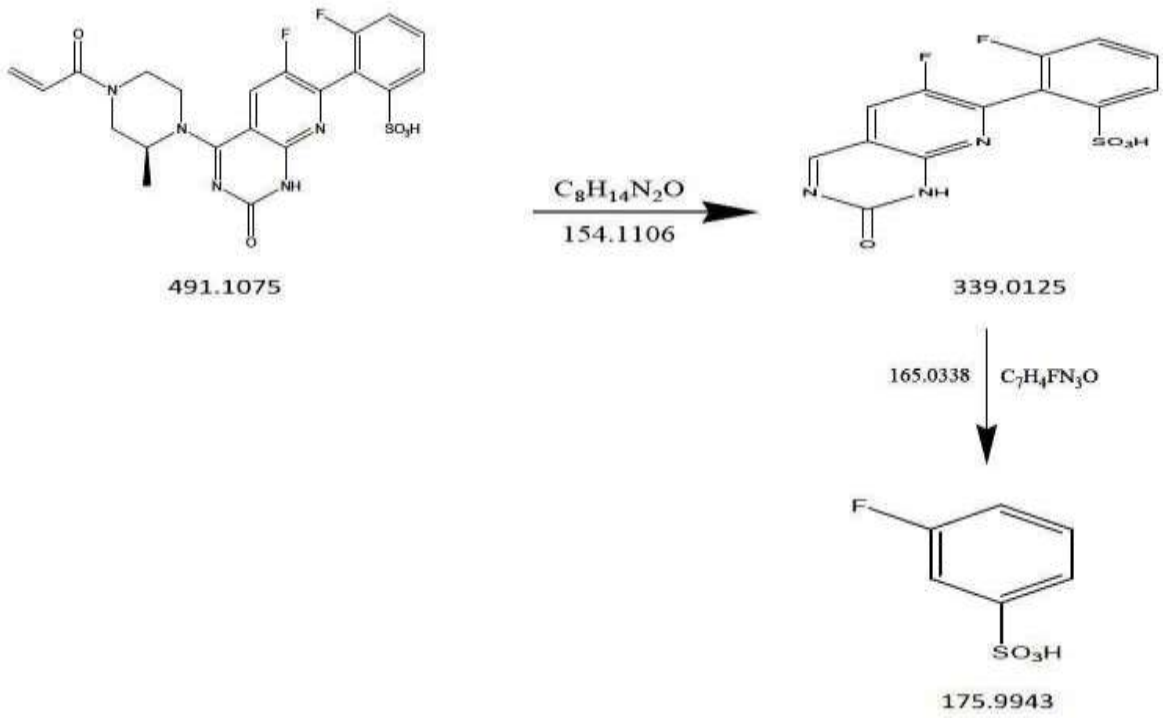


Figu r e 29: Mass Spectra of Reduction Degradation Product (DP-4)



DP4: The fragmentation pathway of DP4 is illustrated in figure 30, and the most intense [M+H]+ ion of m/z-491.1075 was detected under the conditions of reduction degradation, according to the ESI spectra. A large number of product ions were seen in the DP4 MS/MS spectra at m/z-339.0125 (where C8H14N2O was lost from m/z-491.1075) and m/z-175.9943 (where C7H4FN3O was lost from m/z 339.0125).

Figure 30: Fragmentation pathway of Reduction Hydrolysis Degradation Product (DP-4)



All the suggested fragmentation pathway have been validated by the MS/MS tests in conjunction with precise mass measurements.

Figure 31: Mass Spectra of Sotorasib Racemic Mixture

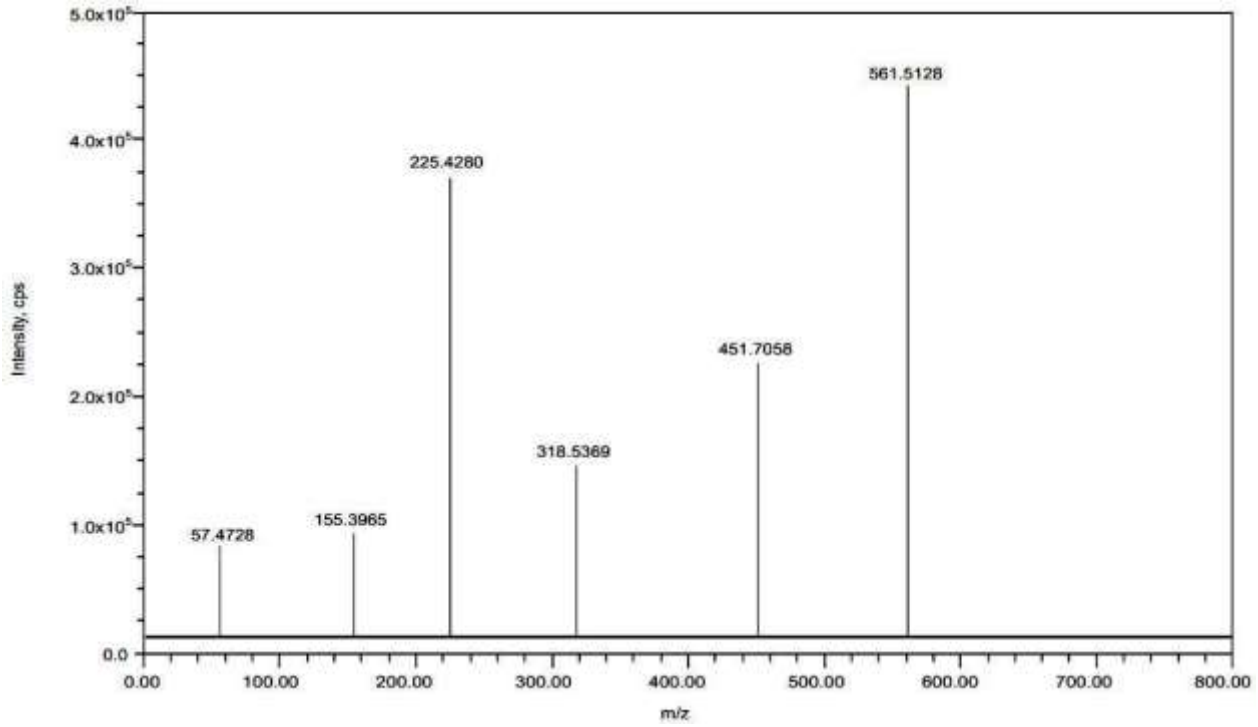
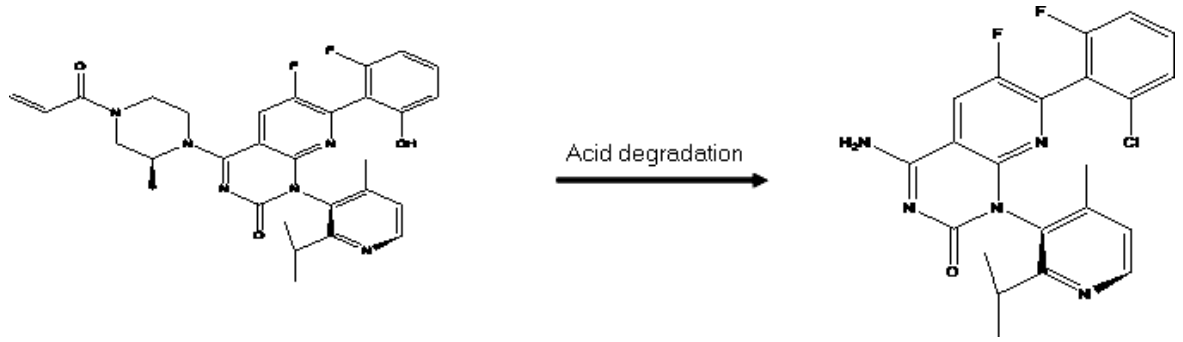


Table: 10 LC-MS/MS data of S-Sotorasib and its degradation products and some major fragments

	Molecular Formula	Calculated Mass(g/mol)	Observed Mass(g/mol)	Error	Retention time(min)	Major Fragment Ions
S-Sotorasib	$C_{30}H_{30}F_2N_6O_3$	560.2347	560.2353	1.070980	2.509	57.0271,155.1112, 225.1279,318.1293 and451.2185
DP1	$C_{22}H_{18}ClF_2N_5O$	441.1168	441.1175	1.586881	4.733	181.0459and 309.0284
DP2	$C_{27}H_{27}F_2N_6NaO_2$	528.2061	528.2072	2.082520	4.318	166.0346,298.0333 and431.1227
DP3	$C_{30}H_{30}F_2N_6O_3$	560.2347	560.2356	1.606470	10.065	155.1112,318.1296 and451.2189
DP4	$C_{21}H_{19}F_2N_5O_5S$	491.1075	491.1084	1.832593	7.207	176.9953and 340.0132

1. Acid degradation reaction of S-Sotorasib with HCl were shown in figure 32

Figure 32: Reaction of S-Sotorasib with HCl

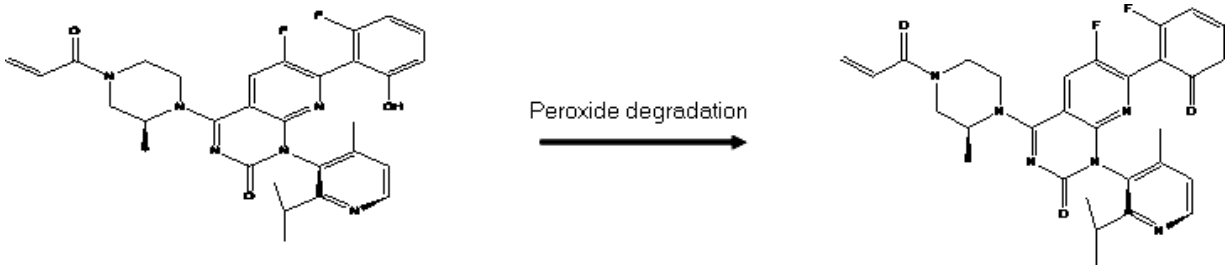


2. Alkali degradation reaction of S-Sotorasib with NaOH were shown in figure 33

Figure 33: Reaction of S-Sotorasib with NaOH

3. Peroxide degradation reaction of S-Sotorasib with H_2O_2 were shown in figure 34

Figure 34: Reaction of S-Sotorasib with H_2O_2



4. Reduction degradation reaction of S-Sotorasib with $NaHSO_3$ were shown in figure 34

Figure 35: Reaction of S-Sotorasib with $NaHSO_3$

CONCLUSION

The developed HPLC method for the estimation of selected drug was simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents were simple to prepare and economical. All the validation parameters within the ICH acceptable range.
The drug was subjected to stress conditions like Acid hydrolysis Alkali hydrolysis, Peroxide degradation Photolytic degradation, Thermal degradation. The obtained DPs were separated and identified and characterized by LC-MS.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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