Stability-indicating RP- HPLC technique for the determination of Sotorasib in pharmaceutical bulk formulations.

Nanduri Gayatri Devi[1], T. Vasundhara[2], B. Ramya Sri[3], K. Mounika[4], R. Swathi[5], M. Keerthi[6], V. NagaLakshmi[7], NVNB Srinivasa Rao[8]

[1,2,3,4,5,6,7]Department of Chemistry, Ch. S. D. St. Theresa's College for Women(A), Eluru-534003,

[8]S.Ch.V.P.M.R Government Degree College, Ganapavaram

Corresponding Author[1]: Associate Professor in Chemistry, Ch. S. D. St. Theresa's College for women(A), Eluru-534003

ABSTRACT

A simple, rapid, precise, sensitive and reproducible reverse phase, high-performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Sotorasib in a pharmaceutical dosage form. Chromatographic separation of Sotorasib was achieved on Waters Alliance-e2695, by using X-bridge phenyl, (150x4.6mm, 3.5µ) column and the mobile phase containing **Tri fluoro acetic acid & Methanol** in the ratio of **50:50% v/v**. The flow rate was 1.0 ml/min; detection was carried out by absorption at **225nm** using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Sotorasib were NLT 2000 and should not be more than **2** respectively. % Relative standard deviation of peak areas of all measurements always less than **2.0.** The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Sotorasib.

Keywords: RP-HPLC, Sotorasib, Relative standard deviation

INTRODUCTION

Chromatographic techniques may be used to detect or quantify a particular analyte since they are selective or specific by nature. To get useful data from the materials being tested, chromatographic methods are used to identify individual components in mixtures containing numerous components.

HPLC and LCMS are the most often used separation techniques in the laboratory. Sample molecules interact specifically with HPLC in both the stationary phase and mobile phase, resulting in chromatographic separation.

There are several applications for this technique in the pharmaceutical industry - in the purification of organic products, natural substances, in quality control labs, research institutions and in clinical chemistry.

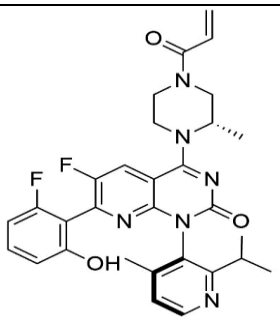
A chromatography technique known as HPLC has been utilized extensively for the isolation and identification of bioactive compounds. A liquid mobile phase and a high-pressure HPLC column are used to separate the analyte from the mixture while it is still in solution. In the column of HPLC instrument, the mixture is uniformly distributed between the stationary phase and the movable phase. The degree of isolation is determined by the amount of contact and affinity between the stationary phase and the solute components. The components with the lowest affinity for the stationary phase will be the first to separate.

The stationary phase is non-polar (column packing is C18, C8, C2, etc.) while the movable phase is polar in the reversed-phase mode (water, methanol, acetonitrile, tetrahydrofuran etc.,). Due to the polar nature of bioactive molecules and the shorter analysis time required by this technique, the bioactive molecules are eluted first and then followed by non-polar chemicals.

Isocratic elution and gradient elution are the two elution modes available in HPLC. According to time programming, the movable phase and composition of a gradient elution run may change from isocratic elution mode to gradient elution mode throughout the run time. As an outcome, isocratic elution is employed in many quality and process control labs, while gradient elution is utilized for more complicated specimens and in the technique development process for mixtures.

Table-1: Drug profile of Sotorasib

IUPAC name	6-fluoro-7-(2-fluoro-6-hydroxyphenyl)-1-(4-methyl-2-propan-2-ylpyridin-3-yl)-4-[(2*S*)-2-methyl-4-prop-2-enoylpiperazin-1-yl]pyrido[2,3-d]pyrimidin-2-one
Molecular Formula	$C_{30}H_{30}F_2N_6O_3$
Molecular Weight	560.6 g/mol
Description	Patients who have had at least one prior therapy for NSCLC and whose tumours contain an aberrant KRAS G12C gene may benefit from sotorasib. This is especially true if cancer has progressed or the patient's condition is too advanced for surgical removal of the tumour.

Therapeutic category	The RAS GTPase family is targeted by sotorasib. For cancers with any KRAS mutation, sotorasib is the first FDA-approved targeted treatment.
Molecular Structure	

MATERIALS AND METHODS

Chemicals: Methanol, trifluoro acetic acid (HPLC grade) and Ortho Phosphoric acid (HPLC grade) purchased from Rankem manufacturers. Water (Milli Q) (HPLC grade) was obtained from in-house production. API of Sotorasib standard was procured from Glenmark, Mumbai.

RP-HPLC Simultaneous Method Development for Sotorasib Equipment:

In this investigation, a photodiode array detector (model 2998) from Waters Alliance was employed in conjunction with a data handling system called Empower 2.0.

Determination of Working Wavelength (λ_{max}):

The isobestic wavelength was utilised to estimate the drug's potency. The isobestic point is the wavelength at which the molar absorptivity of the interconvertible substances is the same. Accordingly, this wavelength was used for the precise calculation of medication quantities.

The wavelength of maximum absorption of the solution of the drug in a mixture of Methanol and 0.1%TFA (50:50) was scanned using PDA Detector within the wavelength region of 200–400 nm against Methanol and 0.1%TFA (50:50) as blank. The absorption curve shows an isobestic point at 225 nm and it was selected as the detector wavelength for the HPLC chromatographic method.

Chromatographic conditions:

Multiple trials were performed to determine the optimal chromatographic settings for this approach.

Preparation of standard stock solution:

Accurately weighed and transferred the 10 mg of Sotorasib working standard into a 10 ml clean dry volumetric flask added diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipetted 1 ml of the above stock solutions into a 10 ml volumetric flask and diluted up to the mark with diluent. (100ppm of Sotorasib)

Sample Solution Preparation:

Accurately weighed and transferred the 22.2mg of Sotorasib sample into a 10mL clean dry volumetric flask added diluent and sonicated it up to 30 mins to dissolve, and centrifuged for 30 minutes to dissolve it completely and made volume up to the mark with the same solvent. Then it is filtered through a 0.45-micron Injection filter. Further pipetted 1ml of the above

stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent. (100ppm of Sotorasib)

0.1% TFA buffer preparation: 1 ml of TFA is dissolved in 1 litre of HPLC water and filtered through a 0.45µ membrane filter.

Preparation of Mobile Phase: Mobile phase was prepared by mixing METHANOL and 0.1%TFA taken in the ratio 50:50. It was filtered through a 0.45µ membrane filter to remove the impurities which may interfere with the final chromatogram.

Preparation of Diluent: Mobile phase was used as a diluent.

Table-2: Optimization of chromatographic condition

Column	X-BRIDGE PHENYL (4.6×150nm,3.5µm)
Movable Phase	METHANOL: 01%TFA (50:50)
Wavelength	225nm
Rate of flow	1ml/min
Volume of injection	10µl
Run period	5min
Observation	This method is suitable for validation

The Sotorasib peak was observed at 3.580 min with peak area 3018100, tailing factor 1.01. This trial was optimized.

METHOD VALIDATION SUMMARY:

Specificity:

The ability of an analytical technique to quantify the target analyte without being affected by background or known contaminants are referred to as "specificity." This was accomplished by recording chromatograms for a blank, a standard, and a sample. To verify that the drug reaction was really specific, we may look at the chromatogram of the blank, which exhibits no response at the retention periods of pharmaceuticals.

LINEARITY:

Preparation of stock solution:

Accurately weigh and transfer 10mg of Sotorasib working standard into a 10 ml clean dry volumetric flask added diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).

Concentrations of 25ppm,50ppm,75ppm,100ppm,125 and 150 ppm of sotarsib were taken and tested for linearity.

Procedure:

Injected each level into the chromatographic system and measured the peak area.

Plotted a graph of peak area versus concentration (concentration on the X-axis and Peak area on Y- the axis) and calculate the correlation coefficient.

Range:

An analytical method's Range is the concentration range across which its accuracy, precision, and linearity have been shown. This range may or may not include the limits of detection.

Inclusion Criteria:

The correlation coefficient should be not less than 0.999.

Precision

Precision is the degree of repeatability of an analytical method under normal operating conditions. Precision is of 3 types

1. System precision
2. Method precision
3. Intermediate precision (Inter-day precision)

To guarantee that the analytical system is functioning correctly, its accuracy is tested using a standard chemical compound. The peak area and percentage drug of six determinations are assessed, and the relative standard deviation (RSD) is computed.

A single batch sample should be tested six times to ensure procedure accuracy. Find out whether a procedure produces the same results with each batch. Six replicates of the sample analysis and a % RSD calculation are shown below.

The precision of the instrument was checked by repeatedly injecting (n=6) solutions of 100ppm of Sotorasib).

Acceptance Criteria:

The % RSD for the absorbance of six replicate injection results should not be more than 2%.

ROBUSTNESS:

To test the method's robustness, the flow rate, mobile phase composition, and temperature were to see how these affected the results. The flow rate varied from 0.9 ml/min to 1.1 ml/min.

Standard solution 100ppm of Sotorasib was prepared and analysed using the varied flow rates along with method flow. On the evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even with the change in the flow rate of ±10%.

The variation of Organic Phase ratio: Standard solution of 100ppm of Sotorasib was prepared and analysed using the varied in mobile phase ratio.

RESULTS AND DISCUSSION

Determination of Working Wavelength (λ_{max}):

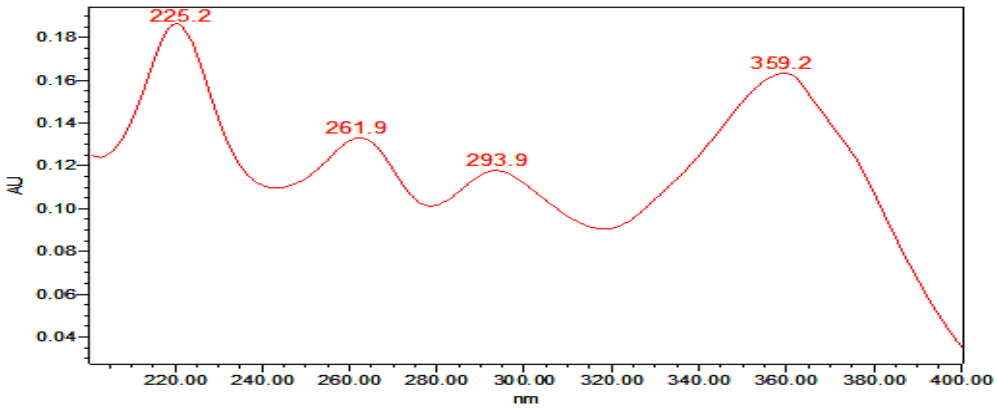


Figure 1: PDA-Spectrum of Sotorasib

Table-3: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Instrument utilized	Waters HPLC with the autosampler and UV detector.
Injection volume	10µl
Mobile Phase	Methanol: TFA(50:50)
Column	Symmetry shield RP18(4.6×150nm,35µm)
Wave Length	225nm
Flow Rate	1 mL/min
Runtime	5min
Temperature	Ambient(25° C)
Mode of isolation	Isocratic mode

Table-4: Chromatogram of Trial-6

S.No	Name	RT	Response	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Sotorasib	3.580	3018100	100.00		1.01	8521

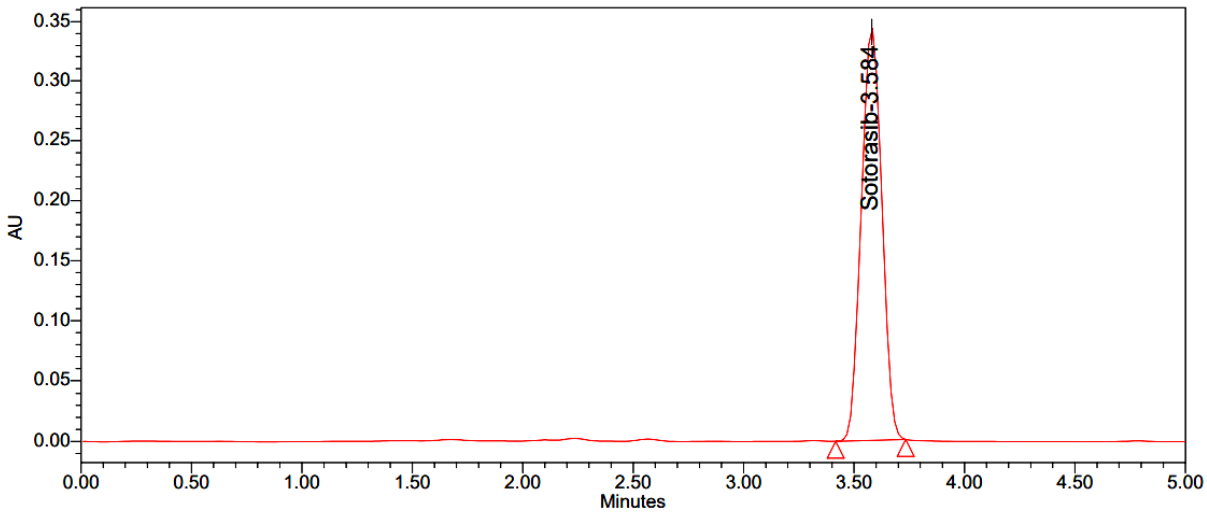


Figure 2: Chromatogram of standard

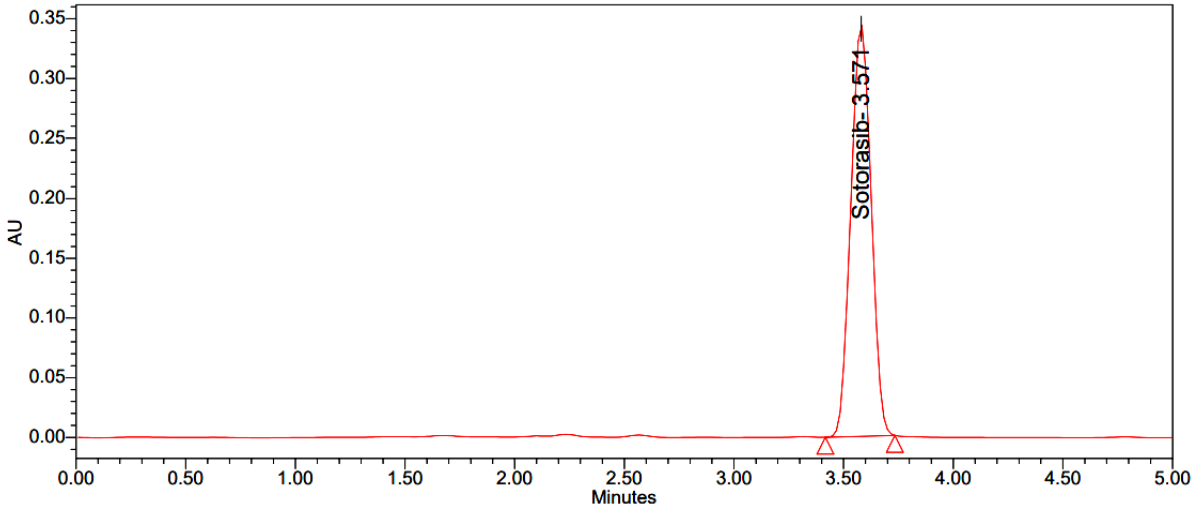


Figure 3: Chromatogram of sample Assay

Table-5: Assay of Sotorasib

Brand	Medication	sample area	Average Area	Std. wt (mg)	Sample wt. (mg)	Label amount (mg)	Std purity	The amount found (µg/ml)	% assay
Lumakras	Sotorasib	3061489 3066955	3064222	10	22.2	120	99.8	10.16	101.6
Sotoxen	Sotorasib	3012689 3037856	3050273	10	29.2	120	99.9	10.02	100.3

Acceptance Criteria:

The % assay should be within the range of 98-102%

Observation: The % assay was found to be within the range.

ANALYTICAL METHOD VALIDATION (HPLC)

The method was validated for its linearity range, accuracy, precision, and specificity. Method validation was carried out as per ICH guidelines.

Linearity:

Table-6: Results of linearity for Sotorasib

S.NO	Sotorasib	
	Conc.(µg/ml)	Peak area
1	25	754135
2	50	1572263
3	75	2290394
4	100	3040525
5	125	3770656
6	150	4584788

Regression equation	y =25304.38x+ 10142.95
Slope	30365.25
Intercept	10142.95
R^2	0.99988

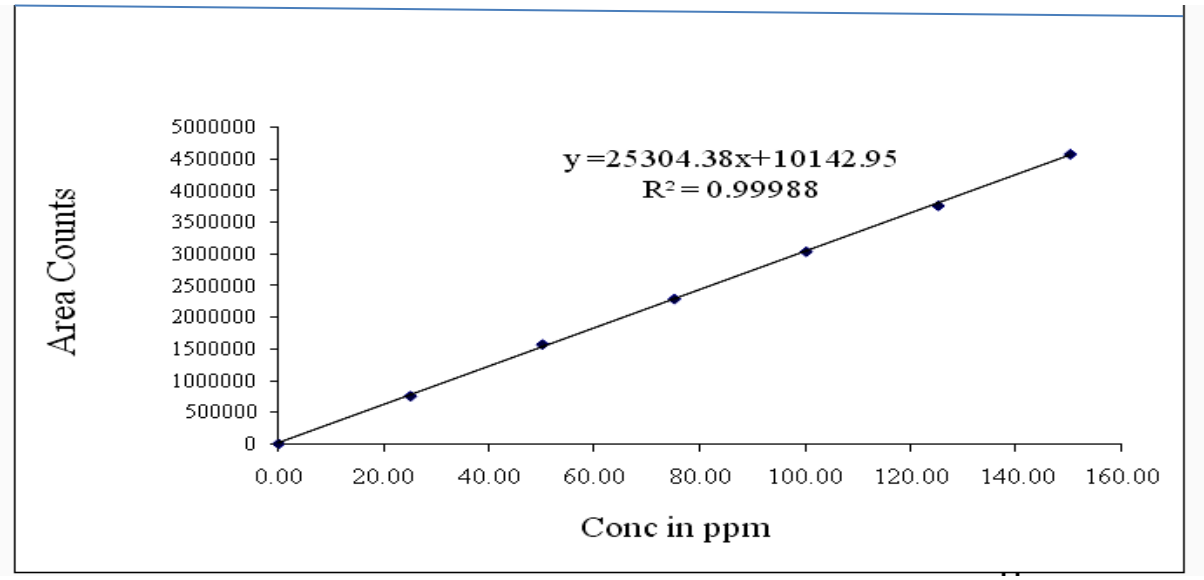


Figure 4: Calibration curve for Sotorasib at 225 nm

Precision:

In a method precision investigation, six distinct standard solutions with sotorasib concentrations of 100 µg/ml each are produced and injected into an HPLC system. It was discovered that the sotorasib %assay was between 98 and 102%. Peak areas, from which mean, SD, and %RSD values were derived, were determined. Table 4 below contains these outcomes.

Table-7: Standard results for Sotorasib by RP-HPLC method

Injection	Area for Sotorasib
Injection-1	3018100
Injection-2	3012025
Injection-3	3012032
Injection-4	3016541
Injection-5	3018100
Injection-6	3026122
Average	3017153
Standard Deviation	5198.69
%RSD	0.17

Table-8: Method Precision for Sotorasib by RP-HPLC method

S. No	Area for Sotorasib
1	3005679
2	3022432
3	3013387
4	3007485
5	3022066
6	3041679
Average	3018788
Standard Deviation	13242.329
%RSD	0.44

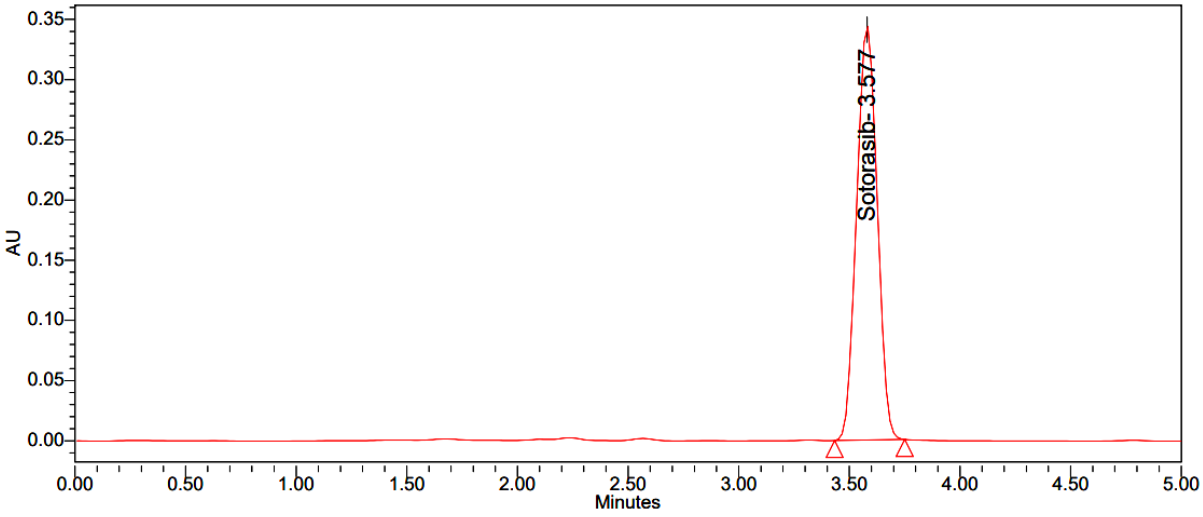


Figure 5: Chromatogram of Method Precision

Acceptance Criteria: The % RSD for the area of six standard injection results should not be more than 2%**.**

Robustness:

Table-9: Robustness results of Sotorasib by RP-HPLC

Parameter	Sotorasib					
	Condition	RT (min)	Peak area	Resolution	Tailing	Plate count
Flow rate Change (mL/min)	Less flow(0.9ml)	3.955	3241736		1.06	8569
	Actual(1ml)	3.580	3018100		1.01	8521
	More flow(1.1ml)	3.277	2992709		1.00	8469
Organic Phase change	Less Org (45:55)	4.814	3447986		1.10	8598
	Actual(50:50)	3.584	3012025		1.04	8525
	More Org (55:45)	2.847	2771340		1.02	8436

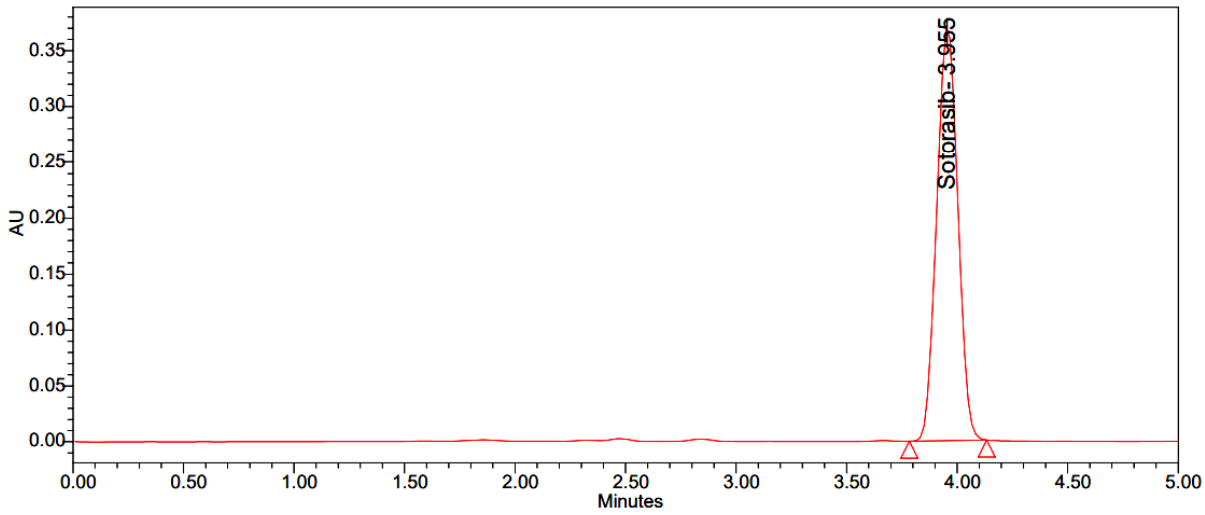


Figure 6: Chromatogram for less flow rate (0.9 ml)

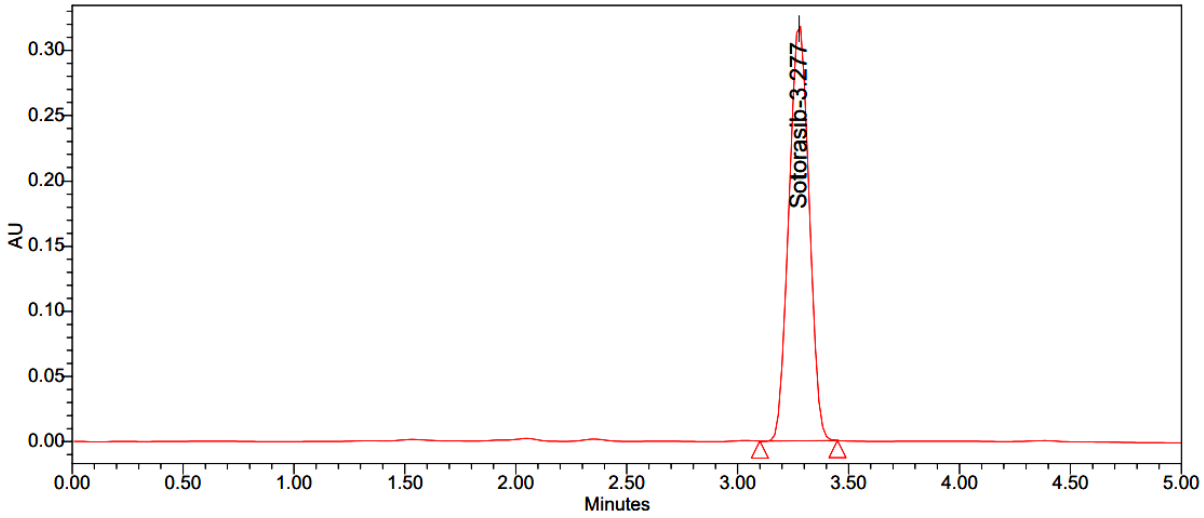


Figure 7: Chromatogram for more flow rate (1.1mL)

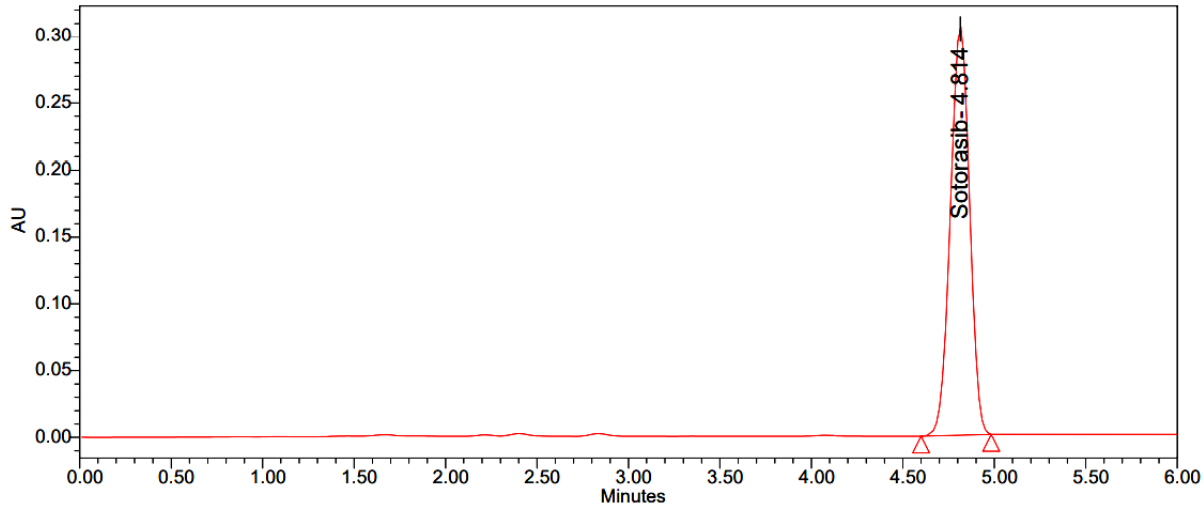


Figure 8: Chromatogram for more Organic Phase (55:45)

CONCLUSION:

The devised HPLC technique for estimating the target drug is easy to implement, quick to complete, highly reliable, and cheap. Preparing the mobile phase and solvents is quick and easy, and they are cheap, dependable, sensitive, and save time.

There was excellent agreement between the recoveries from the samples and the claims made for each medicine on the label, and the results also indicated that formulation excipients did not affect the estimate.

It is deduced that the simple and short proposed methods be most useful for analysis purposes because the system validation parameters of the HPLC method used for the estimation of the selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of excipients as well).

We found that the stability indication test technique using RP-HPLC was straightforward, reproducible, sensitive, and specific, with no cross-contamination from placebo or degradation products. The results of these tests are suitable for regular Sotorasib analysis.

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