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DEVELOPMENT AND VALIDATION OF A NEW HPLC METHOD FOR THE DETECTION OF SONIDEGIB IN MOBILE PHASE AND HUMAN PLASMA

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Keywords:

Sonidegib, HPLC, Mobile phase, Human Plasma, Linearity, Method validation

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ABSTRACT: The main aim of the present research work is to develop a sensitive, precise and accurate HPLC (High-Performance Liquid Chromatography) procedure for the selective estimation of sonidegib in both human plasma and mobile phase. An isocratic separation of sonidegib through a 5µ Zorbax C-18 analytical column with the dimensions of 25 cm × 4.6 mm utilizing mobile phase composition of methanol, water and acetonitrile at a ratio of 10:10:80 v/v. The detection of the analyte was processed at the maximum wavelength of 254 nm and with 1 ml/min flow of the mobile phase. The drug was eluted from the column at the retention time of 3.6 min in plasma samples and 4.81 min in movable phase. Five variable concentration levels of 5, 10, 15, 20, and 25 µg/ml were used for the estimation of recovery and linearity. The recovery findings were 5.03, 9.93, 14.96, 20.14, and 24.91 µg/ml, respectively, for Sonidegib in the mobile phase. Similarly, 6 concentration levels of 20, 40, 200, 400, 800, and 1200 µg/ml were utilized for recovery study, and the findings were 20.15, 40.21, 199.62, 398.16, 798.81 and 1197.28 µg/ml respectively for Sonidegib in plasma. The % RSD findings were found to be <2%, and the correlation coefficient was more than 0.999. The developed method can be useful in bioavailability and bioequivalence studies.

INTRODUCTION: Sonidegib chemically designated as *N*-[6-[(2*S*,6*R*) -2,6-Dimethylmorpholin -4-yl]pyridin-3-yl]- 2- methyl-3-[4-(trifluoromethoxy) phenyl] benzamide with molecular formula of $C_{26}H_{26}F_3N_3O_3$ and molecular weight of 485.498 g/mol. It is utilized to treat basal cell carcinoma, which has relapsed radiation therapy or after surgery in adult patients.

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It effectively obstructs the regulator called smoothened (SMO), inhibiting the hedgehog path from functioning. As a consequence, cancers that depend on the hedgehog path were incapable of grow [1-3].

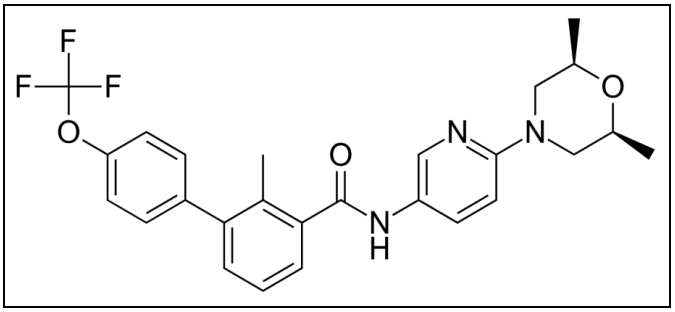

FIG. 1: CHEMICAL STRUCTURE OF SONIDEGIB

The drug prevents a transmembrane protein called SMO, which plays an important role in the hedgehog (Hh)-signal transduction. This results in the prevention of Hh-signalling and anti-tumor activity in several animal models [4, 5].

The literature of the drug revealed that no HPLC methods were reported for the quantification of Sonidegib [6, 7]. The main aim of the present study was to develop and validate an RP-HPLC method having a simple and rapid sample preparation protocol for the quantification of sonidegib in both plasma and mobile phases. The developed method can be applicable for bioavailability and bioequivalence studies.

MATERIALS AND METHODS:

Chemicals: Sonidegib standard was procured from sun pharmaceuticals, Mumbai, India. HPLC-grade acetonitrile and methanol were obtained from Merck, Mumbai, India. In the present work distilled water (HPLC-grade) was acquired from Lichrosolv (Mumbai, India). Pooled drug-free frozen human plasma (K2-EDTA as an anticoagulant) was obtained from St. Theresa Blood Bank, Hyderabad, which was used during validation and study sample analysis. The plasma was stored at -70 ± 5 °C.

Apparatus and Equipment: The instruments utilized in the present study were Sigma-200 electronic balance, PCi-3.5 L-sonicator, Universal hot-air oven, and Unilab's digital pH meter. Additionally, syringe [Hamilton (Rheodyne-20 µl)] and syringe filter (Himedia-Syringe driven 0.22 µ filters) were used.

Preparation of Mobile Phase and Stock Solutions: Mobile phase mixture was processed with HPLC-grade acetonitrile, methanol, and water in the proportion of 80:10:10. The movable phase was processed for filtration through a 0.22 µm size nylon filter. The processed movable phase was subjected to degasification by sonication.

Sonidegib 1000 µg/ml stock solution in the movable phase was processed and subjected for sonication to dissolve and proper mixing for 15 min. Dilutions of 100, 50, 15, and 1 µg/ml were processed by utilizing freshly prepared movable phases. The resulting solutions were subjected to sonication for degasification separately.

In a similar manner sonidegib 1000 µg/ml stock solution was processed in human plasma. To prepare this solution, 10.0 mg of sonidegib drug was mixed with 1.0 ml of human plasma and 2.0 ml methanol (as de-proteinizer). The resulted mixture was vortexed first for 15.0 min and subjected for centrifugation at 5000 rpm for 5.0 min. The clear supernatant liquid was transferred into a vial for further drying for 45 min. The resulting residue was combined with 20 µL movable phase. The resulting plasma solution was filtered through 0.22 µ nylon filter before infusing into the chromatographic system. Serial dilutions were processed from the stock solution to get the 10 µg/ml solution.

Liquid Chromatography: Liquid chromatographic system consisting of Shimadzu-HPLC model containing SPD-20A UV/visible detector, LC-20AD pump and injector (Rheodyne) with 20µl fixed loop utilized for the elution. Separation was processed on column Zorbax C-18, 5 micrometer, column dimensions: 25cm × 4.6mm at ambient temperature. The acquisition chromatograms were done with LC-solutions software. The movable phase comprising of acetonitrile, methanol and water at a proportion of 80:10:10 v/v respectively. Sonidegib was detected at 254 nm with the flow rate of 1 ml/min in both mobile phase and plasma. The processed samples were infused using a 20 µl fixed loop. All the estimations were processed at an ambient temperature with 8 min run time. The optimized conditions of liquid chromatographic technique was represented in **Table 1**.

TABLE 1: OPTIMIZED ANALYTICAL PARAMETERS FOR SONIDEGIB

Parameters	Conditions
Column	Zorbax C-18, (5 µ, 250mm × 4.6mm)
Mobile phase	Acetonitrile, methanol and water in a proportion 80:10:10 v/v
Flow rate	1ml/min
Run time	8 min
Volume of injection loop	20 µl
Detection wavelength	254 nm
Temperature of the Column	Ambient
Retention time	4.81 min in mobile phase and 3.6 minutes in plasma

Method Validation: The developed technique was validated by assessing selectivity, linearity, robustness, accuracy, precision, detection limit, quantification limit, and solution stability as per the ICH Q2B guidelines [8-21].

Specificity and Linearity Curve: Differentiation of analyte from the other substances like impurities and excipients form the sample was processed by performing the specificity. It was determined by infusing 6 duplicate injections of movable phase and dosage form to identify the intrusion between the placebo and sonidegib chromatograms.

The method linearity was processed to find out the correlation between the concentration of the drug component and its detector response. Linearity solutions at different concentration levels were processed in movable phase from the stock solution to get 5.0, 10.0, 15.0, 20.0, and 25.0 µg/ml standard concentrations, and the calibration plot was developed [8, 15].

While for sonidegib in human plasma, the linearity dilutions of 20, 40, 200, 400, 800, and 1200 µg/ml solutions were processed to evaluate the linearity. The correlation coefficient, slope, and intercept [19] were calculated to form the statistics Sonidegib in both movable and plasma samples.

Inter-day & Intra-day Precision: Method precision was processed in terms of inter-day and intra-day precision for 3 subsequent days. Five linearity concentrations were utilized to estimate precision. In the case of intra-day precision, 5 dissimilar concentrations were 5, 10, 15, 20, and 25 µg/ml in movable phase and 6 dissimilar concentrations of 20, 40, 200, 400, 800, and 1200 µg/ml in plasma were applied.

System Suitability: This parameter was assessed by injecting the 5 replicates of the same standard concentration in movable phase and similarly 5 replicates of unique standard plasma concentration solution into the liquid chromatographic system. On each day of method validation, 5 successive injections were processed to assess the system suitability.

Solution Stability: Solution stability of the method is necessary for the quantification of drug components. In this parameter shelf-life of the processed solutions was evaluated by storing the stock solution at room temperature for 16.0 h and at -15 to -20 °C for 7 days in a freezer. Five injections of similar concentrations were utilized to estimate the differentiation among experiments.

Sensitivity Determination: The lowest concentration level of analyte that can be detected is called the limit of detection (LOD). The low concentration of 1 µg/ml solution was repetitively injected 5 times and was set as LOD [9, 18]. LOD was calculated based on the formula LOD = 3.3 SD/slope, which was dependent on the regression line. The lowest concentration level of analyte that can be quantified is called the limit of quantification (LOQ) [10]. So, the linearity curve was assessed from 5 to 25 µg/ml for the movable phase and from 20 to 1200 µg/ml for plasma.

RESULTS AND DISCUSSION: The validation parameters like specificity, system suitability, precision, linearity, accuracy, LOD, LOQ, and solution stability were successfully estimated and found to be within the acceptable limits. Five variable concentrations of 5, 10, 15, 20, and 25 µg/ml were processed for the estimation of linearity **Fig. 2**, and their respective recoveries were found to be 5.03, 9.93, 14.96, 20.14, and 24.91 µg/ml for Sonidegib in the movable phase.

Similarly, 6 variable concentrations of 20, 40, 200, 400, 800, and 1200 µg/ml were processed, and their respective recoveries were found to be 20.15, 40.21, 199.62, 398.16, 798.81 and 1197.28 µg/ml for Sonidegib in plasma **Fig. 4** [11, 12]. The retention time of Sonidegib in plasma and movable phase was found to be 3.6 min **Fig. 5** and 4.81 min **Fig. 3,** respectively [10].

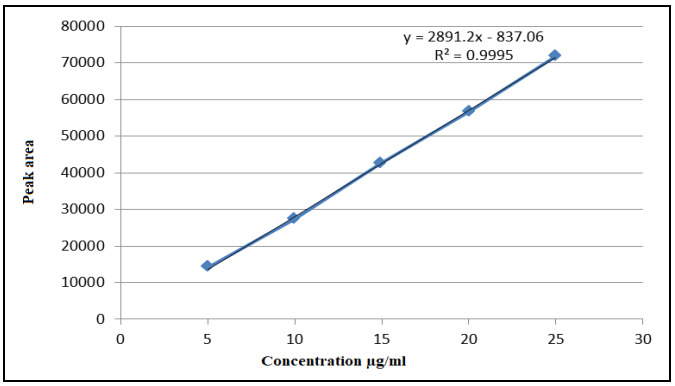

FIG. 2: LINEARITY CURVE OF DIFFERENT CONCENTRATIONS OF SONIDEGIB IN MOBILE PHASE

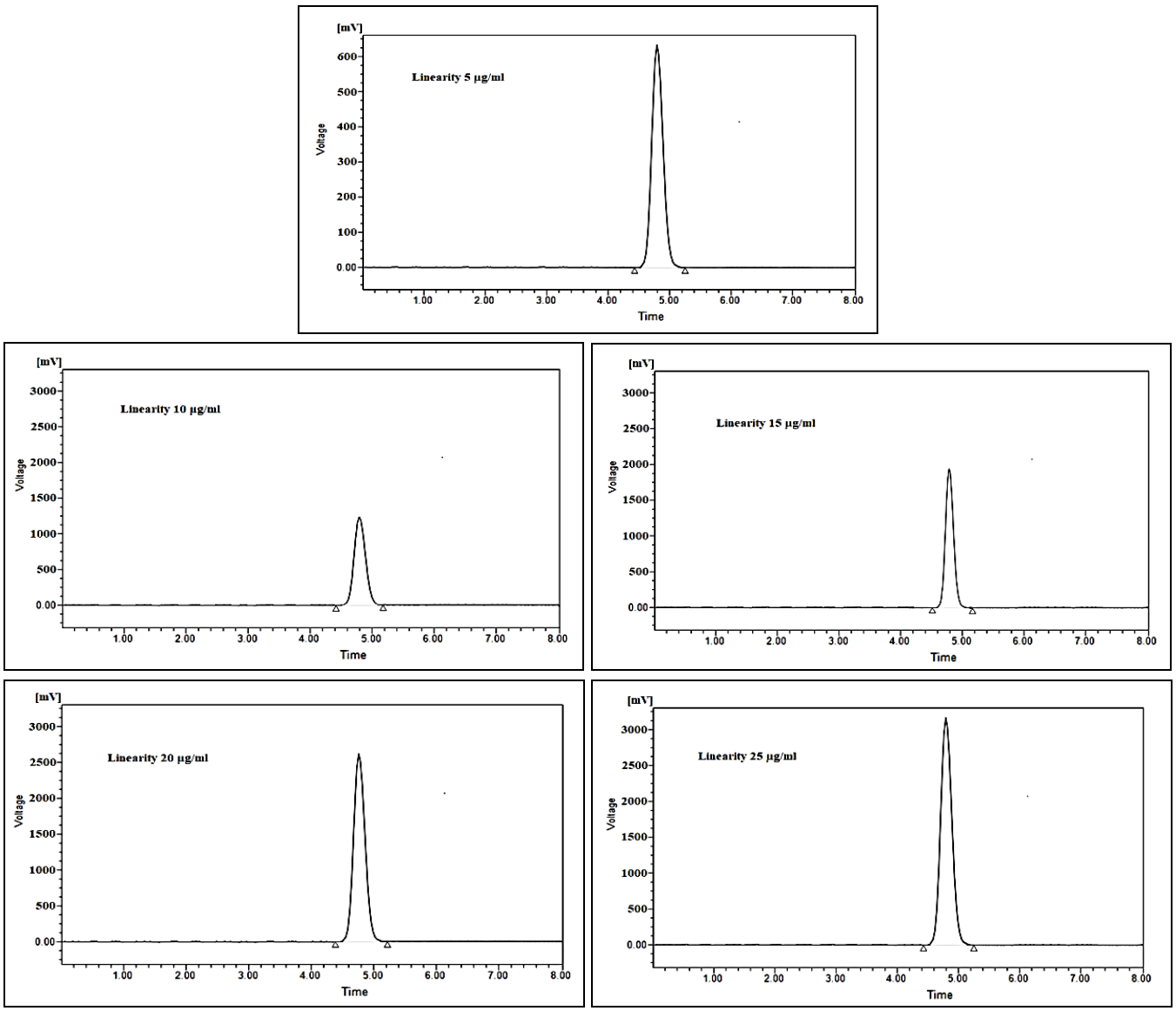

FIG. 3: LINEARITY CHROMATOGRAMS OF DISTINCTIVE CONCENTRATIONS OF SONIDEGIB IN MOVABLE PHASE

Duplicate injections of 5 selected concentration levels of Sonidegib in movable phase of 5, 10, 15, 20 and 25 µg/ml and 6 selected concentration levels of Sonidegib in plasma of 20, 40, 200, 400, 800 and 1200 µg/ml gave the confirmation of accuracy as represented in **Table 2** and **Table 3** respectively.

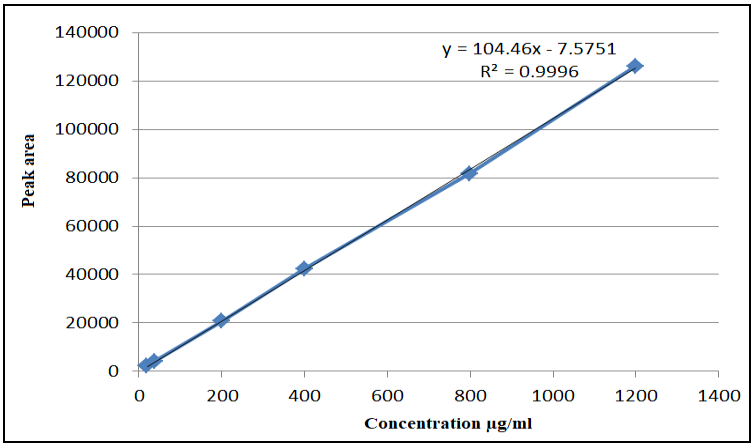

FIG. 4: LINEARITY CURVE OF DIFFERENT CONCENTRATIONS OF SONIDEGIB IN PLASMA

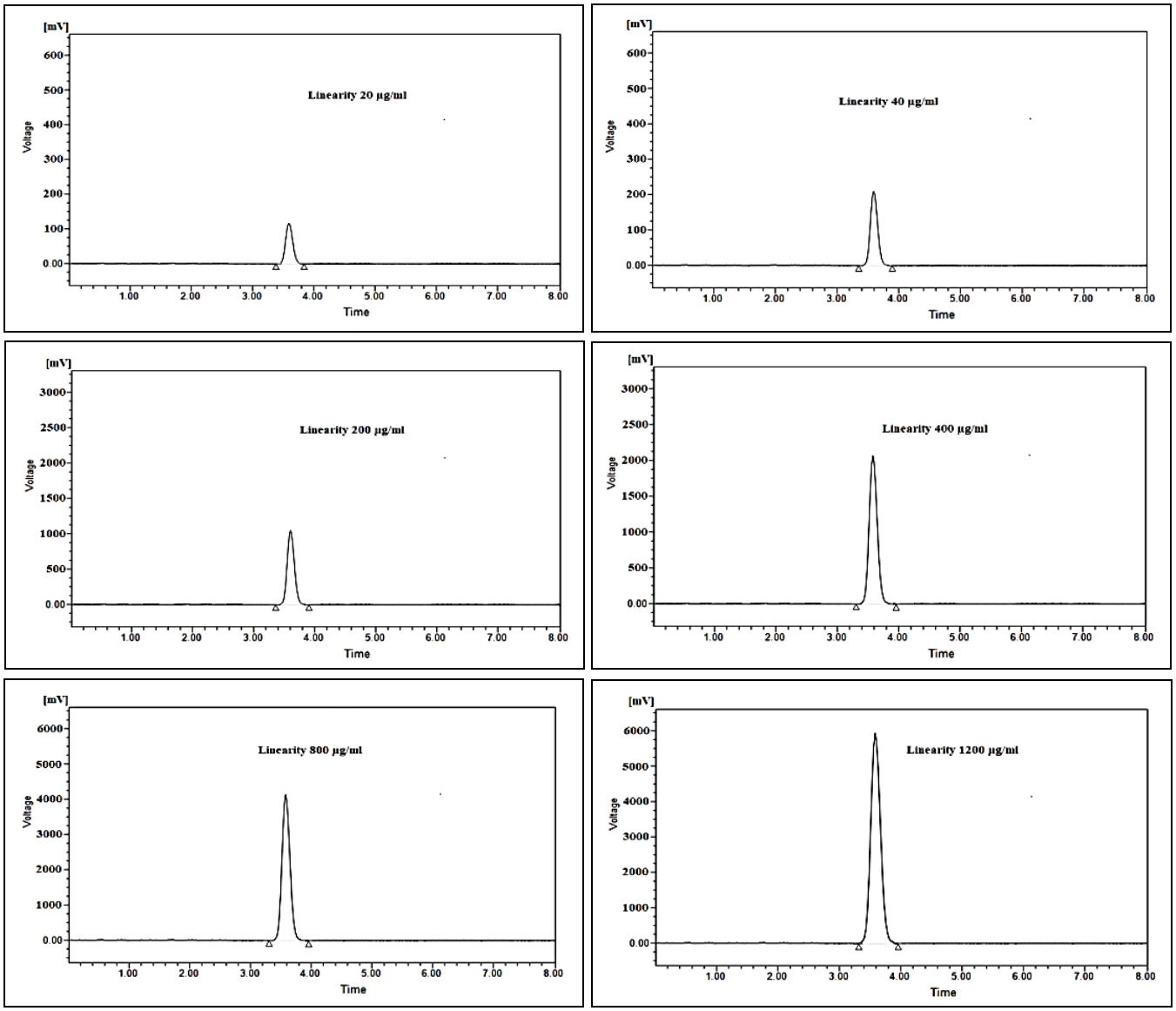

FIG. 5: LINEARITY CHROMATOGRAMS OF DIFFERENT CONCENTRATIONS OF SONIDEGIBIN PLASMA

TABLE 2: OPTIMIZED DIFFERENT CONCENTRATIONS OF SONIDEGIB IN MOBILE PHASE

Concentration(µg/ml)	Sonidegib in Movable Phase	
	Found (µg/ml) Mean ± SD (n = 6)	%Recovery (n = 6)
5	5.02 ± 0.04	101.89
10	9.89 ± 0.021	99.18
15	14.89 ± 0.019	99.68
20	20.16 ± 0.121	100.59
25	24.92± 0.201	99.86

SD: standard deviation

TABLE 3: OPTIMIZED DIFFERENT CONCENTRATIONS OF SONIDEGIB IN PLASMA

Concentration(µg/ml)	Sonidegib in Movable Phase	
	Found (µg/ml) Mean ± SD (n = 6)	%Recovery (n = 6)
20	19.05 ± 0.03	90.54
40	40.04 ± 0.025	100.07
200	199.41 ± 0.071	99.41
400	398.23 ± 0.126	99.69
800	798.18 ± 0.171	100.84
1200	1998.25 ± 0.131	99.83

SD: standard deviation

TABLE 4: PRECISION OF THE PROPOSED METHOD FOR SONIDEGIB IN MOBILE PHASE

		Variables	5	10	15	20	25
Sonidegib in Mobile Phase	Inter-Day	Mean	4.95	9.57	15.067	19.36	24.75
		(n=6) ± SD	±0.006	±0.008	±0.081	±0.29	±0.148
		RSD (%)	0.74	0.078	0.342	0.781	0.164
	Intra-Day	Mean	4.94	9.416	14.73	19.327	24.94
		(n=6) ± SD	±0.015	±0.014	±0.445	±0.394	±0.146
		RSD (%)	0.72	0.049	1.21	0.894	0.162

SD: standard deviation; RSD: Relative standard deviation

TABLE 5: PRECISION OF THE PROPOSED METHOD FOR SONIDEGIB IN PLASMA

Sonidegib in Plasma	Variables	20	40	200	400	800	1200
Inter day	Mean (n=6)	19.48	39.48	199.038	399.01	799.19	1999.5
	± SD	±0.006	±0.007	±0.097	±0.42	±0.125	±0.142
	RSD (%)	0.074	0.014	0.096	0.148	0.031	0.0155
Intra day	Mean (n=6)	19.54	39.59	199.42	399.21	799.62	1998.7
	± SD	±0.007	±0.006	±0.451	±0.214	±0.135	±0.214
	RSD (%)	0.0635	0.014	0.494	0.150	0.031	0.037

SD: standard deviation; RSD: Relative standard deviation

Duplicates of 5 concentration levels of 5, 10, 15, 20, and 25 µg/ml of Sonidegib in movable phase on day-1and for 3 succeeding days were processed for the evaluation of precision. The %RSD findings for inter-day precision were 0.74, 0.078, 0.342, 0.781 and 0.164 in respective concentrations. The % RSD findings of intra-day precision were 0.72, 0.049, 1.21, 0.894 and 0.162. %RSD values were found to be < 2.0% as represented in **Table 4**. Duplicates of 6 concentration levels of 20, 40, 200, 400, 800, and 1200 µg/ml of Sonidegib in plasma on the day-1and for 3 succeeding days were processed for the evaluation of precision. The %RSD findings of inter and intra-day precision were found to be <2.0% for Sonidegib in respective concentrations. The findings were tabulated in **Table 5**.

System suitability of analyte was processed, and the parameters like retention time, coefficient of correlation, and peak area were represented in Table 6. All the validation data were processed as per the ICH guidelines and were within the acceptable limit throughout the study. Mean concentration and percentage recovery of Sonidegib at room temperature and in the freezer were found within the limit and were shown in **Table 7**.

TABLE 6: STATISTICAL ANALYSIS OF SONIDEGIB CONCENTRATIONS WITH THEIR SYSTEM SUITABILITY TESTS

Drug	Consideration	Mean
Sonidegib in mobile phase	Retention Time	4.81 min
	Area	1986 at 15 µg/ml
	Coefficient of correlation	0.9995
	Linearity Equation	y = 2891.2x - 837.06
Sonidegib in plasma	Retention Time	3.6 min
	Area	20716 at 200 µg/ml
	Coefficient of correlation	0.9996
	Linearity Equation	y = 104.46x - 7.5751

TABLE 7: AVERAGE CONCENTRATION AND % RECOVERY OF SONIDEGIB AT AMBIENT AND FREEZE TEMPERATURE

Drugs	Experimental Parameters	Amount added (µg/ml)	Measured Concentration (µg/ml) n=6	% Recovery
Sonidegib in movable phase	Room temperature (16h)	15	14.82	98.80
	-15 to -20°C (7 days)	15	14.91	99.40
Sonidegib in plasma	Ambient temperature (12h)	200	198.69	99.34
	-15 to -20°C (7 days)	200	199.01	99.50

CONCLUSION: Validation parameters like specificity, Linearity, precision, system suitability, accuracy, LOD, LOQ, and solution stability were successfully estimated and found to be within the acceptable limits as per the ICH guidelines for the developed technique. LOD and LOQ values were calculated from the linearity curve for Sonidegib analysis in plasma and in the movable phase. Five variable concentration levels of 5, 10, 15, 20, and 25 µg/ml were used for the estimation of recovery and linearity. The recovery findings were 5.03, 9.93, 14.96, 20.14, and 24.91 µg/ml respectively for Sonidegib in the mobile phase. Similarly, 6 concentration levels of 20, 40, 200, 400, 800, and

1200 µg/ml were utilized for recovery study, and the findings were 20.15, 40.21, 199.62, 398.16, 798.81 and 1197.28 µg/ml respectively for Sonidegib in plasma. Moreover, the developed technique has the intended appliance in pharmacokinetics, pharmacodynamics, and clinical pharmacology studies.

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CONFLICTS OF INTEREST: We declare that we have no conflict of interest.

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