RESEARCH ARTICLE

Method Development and Validation for the Determination of Sotorasib by LC-MS/MS Technique

Phani K Sunkara, Sreedhara Chaganty, K Ramakrishna*

Department of Chemistry, Institute of Science, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India.

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ABSTRACT

The main of the research work is to develop and validate a linear, precise and specific LC-ESI-MS/MS method for the quantification of sotorasib. Chromatographic resolution was achieved with ODS Zorbax (50 × 4.6 mm, 2.1 µ) C18 column and a mobile phase composition of methanol, 0.1% formic acid and ACN in the proportion of 50:15:35 with 0.5 mL/min flow of the mobile solvent system from the stationary column procedure was executed by monitoring the established ionic transitions of m/z- 561.09/316.84 for sotorasib and 478.09/451.08 for apalutamide internal standard in multiple reaction monitoring. The linear plot regression line was y = 0.0001x + 0.0011, with a correction coefficient (r2) of 0.9996. The %CV outcomes for matrix effect at low-QC and High-QC levels were 2.90 and 3.41%, respectively. The percentage average recoveries for sotorasib in high-QC (11.25 µg/mL), MQC (7.50 µg/mL) and low-QC (1.05 µg/mL) were 102.41, 98.07, and 102.41%, respectively. The obtained values were between 2.00 and 4.03% for the QC (0.375, 1.05, 7.50 and 11.25 µg/mL). The established technique was subjected for validation as per the food and drug administration (FDA) guiding principles and can be useful for the evaluation of sotorasib in biological samples in quality control, forensic and bioavailability studies.

Keywords: Sotorasib, Lung cancer, Liquid chromatography tandem mass spectrometry, Validation, Accuracy.

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INTRODUCTION

Sotorasib, IUPAC name is represented as 6-fluoro-7-(2-fluoro-6-hydroxy phenyl)-(1 M)-1-[4-methyl -2-(propan -2-yl) pyridine -3-yl] -4-[(2S)-2- methyl -4-(prop-2-enoyl) piperazin-1-yl] pyrido [2,3-d] pyrimidin-2(1H)-one with empirical formula of C30H30F2N6O3 (Figure 1). Sotorasib is approved for treating people who have previously been treated with at least one other systemic therapy for KRAS G12C-mutated locally metastatic or advanced non-small cell lung cancer (NSCLC). In a normal state, GTP will bind to KRAS and cause the protein to become active. This will also promote the effectors that are involved in the MAP kinase pathway. GDP is produced from GTP by hydrolysis, which inactivates KRAS. The presence of KRAS G12C mutations inhibits the hydrolysis of GTP, hence preserving its active state.[1,2]

The anti-cancer medicine sotorasib, marketed under the brand names Lumakras and Lumykras, is typically taken by patients diagnosed with NSCLC. It zeroes down on a particular mutation, known as G12C, in the protein K-Ras, which is encoded by the KRAS gene and is the driving force behind many types of cancer. The protein is kept inactive by sotorasib's ability to bind to the cysteine residue present in KRAS G12C mutations. Because the cysteine residue that sotorasib targets is not present in the wild type of KRAS, any off-target effects caused by the drug are eliminated. This mutation may be found in 13% of patients with non-small cell lung cancer, 3% of patients with colorectal cancer and appendix cancer, and 1 to 3% of patients with solid tumors.[3,4]

Literature survey on sotorasib drug reveals that HPLC[5,6]and LC-MS/MS[7-9] quantification methods for the analysis of sotorasib was reported. So, an LC-ESI-MS/MS analytical method is needed to estimate sotorasib with less retention time in the biological matrix.

MATERIALS AND METHODS

Reagent Chemicals

Sotorasib and apalutamide (IS) were gift samples from Glenmark, Mumbai, India. LC grade ACN (acetonitrile) and methyl alcohol were acquired from J. T. Bakers, Hyderabad. Water was utilized for total research work from water purification (Milli Q) systems. Formic acid of analytical grade was obtained from Merk Pvt. Ltd., Mumbai, India.

Equipment

The Applied Biosystem Sciex-API-4000 Tandem mass spectrometer was combined with an auto-sampler

*Author for Correspondence: karipeddirk@gmail.com

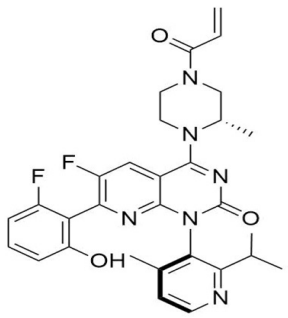

Figure 1: Sotorasib chemical structure

equipped Shimadzu LC20ADvp (Shimadzu, Japan) liquid chromatographic system. The solvent pump is a Japanese-made Shimadzu LC20AD module. Analyst software (version 1.4.2: Applied Biosystems) was used to combine all of the chromatographic results.

Quality and Calibration Standard Solution Preparation

A 100 µg/mL of sotorasib and apalutamide **(**IS**)** stock solutions were processed by solubilizing in the calculated quantity of mobile phase. Standard and quality control standards were processed with blank plasma sample from the standard stock solution of sotorasib. Eight linear standard levels of different concentrations were processed by spikes of the blank plasma with sotorasib standard solution create eight calibration standards at concentrations of 0.375, 0.600, 1.050, 1.900, 4.000, 7.000, 11.000 and 15.000 µg/mL. Low (Low-QC), median (Median-QC) and high (High-QC) standards were quality control (QC) samples, processed by spiking blank plasma with sotorasib to made solutions of 1.050, 7.500 and 11.250 µg/mL, respectively. From apalutamide stock solution of 500 ng/mL its working solution was processed with 70% acetonitrile. Processed samples were kept at 20°C up to the assessment of the samples.

Parameters of Liquid Chromatography

The chromatographic resolution was achieved with ODS Zorbax (50 × 4.6 mm, 2.1 µ) C_{18} column and a mobile phase composition of methanol, 0.1% formic acid and ACN in the proportion of 50:15:35 with 0.5 mL/min flow of the mobile solvent system from the stationary column. Auto-sampler and column oven temperatures were adjusted to 5 and 40°C separately.

Mass Spectrometric Parameters

Parameters of the mass system were improved as follows: 30 psi, nebulizer gas (N2); 450°C source temperatures; 30 psi, curtain gas (N2); 25 psi, heater gas (N2); CAD gas 8 (N2); 4500V ionspray voltage; 12V, entrance potential; 65V, declustering potential; 0.6 mL/min, source flowing rate with no split and collision energy (CE) 18V for both the sotorasib and apalutamide, collision cell exit potential-14V and dwell time 200 ms for the sotorasib and collision cell exit potential-15 V for IS. Product and parent ions detection was executed in MRM mode, with transition pairs of m/z- 561.09/316.84 for sotorasib and 478.09/451.08 for apalutamide internal standard.

Protocol for Sample Preparation

To 250 µL of plasma (spiked with sotorasib to required concentration), 100 µL of apalutamide (1-µg/mL) were mixed in order to precipitate the proteins from the sample, 4 mL of methanol was added to it, and then it was centrifuged at 5000 rpm for 15 to 20 minutes while it was kept at 5°C. The lyophilizer was used to dry out the organic component. After adding 250 µL of movable solvent to the residue, sufficient samples were transferred into labeled Autosampler vials and then injected into an LC-MSMS system.[10, 11]

Validation

The linearity, accuracy, lower limit of quantification (LLoQ), range, recovery, selectivity, precision, and matrix effect of the quantitative determination of sotorasib in plasma were tested in accordance with the guidelines for bio-analytical technique validation in pharmaceutical development. This was done in order to validate the quantifiable assessment of sotorasib in plasma.[12-14]

RESULT AND DISCUSSION

The chromatograms produced throughout the validation procedure passed the assessment and the blank, blank + IS, and LLoQ chromatograms resulted in Figures 2 and 3, respectively.

Specificity

A 375 ng/mL sample was injected into the LC-MS/MS system under optimal LC conditions to separate sotorasib from plasma contaminants and endogenous components. By measuring plasma noise, technique specificity was assessed.[15,16] Interference peak area should be < 20% of LLoQ peak area and < 5% of mean IS peak area(Figure 2). The LLoQ concentration should not fluctuate more than 20% from nominal concentration (NC).

Linear Plot

Calibration plot of the analytical technique was processed by constructing the standard plot between concentration values and peak response fractions of sotorasib to IS. Calibration standards ranging from 0.375 to 15.0 µg/mL (Figure 4).[17, 18] The linearity curve regression equation was y = 0.0001x + 0.0011, with the linear curve findings shown in Table 1.

Table 1: Sotorasib linearity data

LS-ID	*Concentration (ng/mL)*	*Average response*	*IS response*	*Analyte/IS response*
LS -1	375	4804	123864	0.038784
LS -2	600	7758	124012	0.062558
LS -3	1050	13023	122954	0.105918
LS -4	1900	26828	123957	0.21643
LS -5	4000	53867	123367	0.43664
LS -6	7000	89937	123158	0.730257
LS -7	11000	142657	123995	1.150506
LS -8	15000	196974	123002	1.601389

CS: Calibration standard.

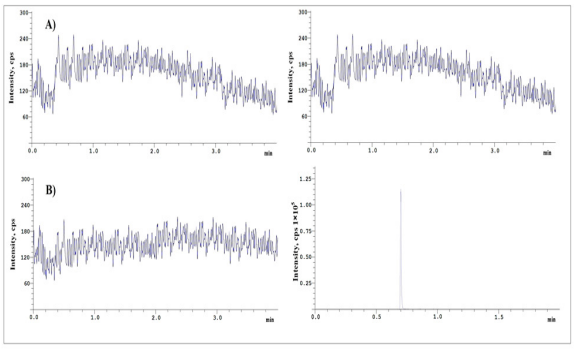

Figure 2: (a) Chromatograms of blank plasma, (b) blank plasma with IS.

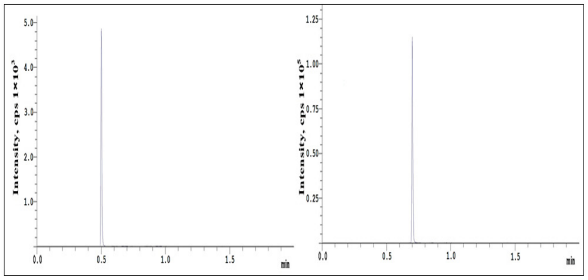

Figure 3: Representative chromatograms of plasma spiked with sotorasib at LLoQ and IS.

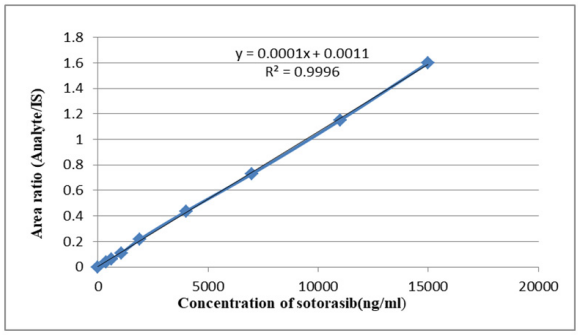

Figure 4: Calibration curve of sotorasib

Inter and Intraday Precisions

Accuracy and precision were determined in the form of inter and intraday. Five replicates per day were infused with the QC standards (1.05, 7.50 and 11.25 µg/mL) and LLoQC (375 ng/mL) to determine the intraday precisions and accuracies. On 5 separate days, we analyzed our quality control standards (1.05, 7.50 and 11.25 µg/mL) and LLoQC (375 ng/mL) to determine our inter-day precision and accuracy. Except for LLoQ QC, where it should not exceed 20%, the within- and between-batch %CV values for high, medium, and low concentrations should be under 15%. The analytical method's intraday and interday precision was assessed by looking at the %CV figures. The results for the quality control samples ranged from 2.00 to 4.03%. There was no significant variation between any of the results (15%). Table 2 displays the tabulated findings.

Extraction Recoveries

Peak areas of extracted samples of sotorasib were compared to those of sotorasib at a control concentration for tests of recovery. The %average recovery outcomes for sotorasib in high-QC (11.25 µg/mL), MQC (7.50 µg/mL) and low-QC (1.05 µg/mL) were 102.41, 98.07 and 102.41%, respectively (Table 3 and Figure 5).

Table 2: Sotorasib Intra and interday precision data

QC	*Sotorasib (µg/mL)*			
Intra batch	*Average*	*SD*	*%CV*	*%Bias*
LLoQ	367	14.82	4.038	-2.13
Low-QC	1017	24.74	2.432	-3.14286
Median-QC	7721	298.31	3.863	2.94
High-QC	11364	395.74	3.482	1.01
LLoQ	384	13.52	3.520	2.40
Low-QC	1065	21.37	2.006	1.43
Median-QC	7745	285.94	3.691	3.26
High-QC	10914	401.84	3.681	-2.98
LLoQ	361	12.85	3.559	-3.73
LLoQ	1069	22.65	2.118	1.80
Low-QC	7621	278.91	3.659	1.61
Median-QC	11109	411.37	3.703	-1.25
Inter batch	Average	SD	%CV	%Bias
LLoQ	359	13.54	3.771	-4.26
Low-QC	1085	26.82	2.471	3.33
Median-QC	7408	298.38	4.027	-1.22
High-QC	11324	446.95	3.941	0.65

Table 3: Recovery of sotorasib after extraction

Concentrations level	X	Y	% Recovery	%Mean recoveries	% RSD
LQC	13868	13267	95.67	98.72	2.82
MQC	99074	97161	98.07		
HQC	148597	152178	102.41		
IS	123575	122252	98.93		

X, mean recoveries of unextracted samples; Y, mean recoveries of extracted samples.

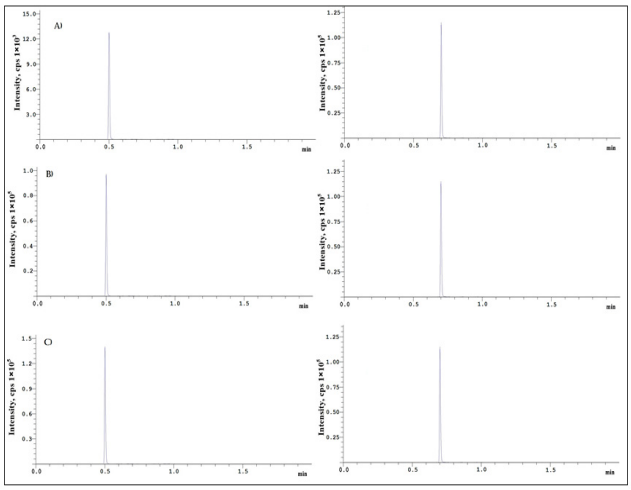

Figure 5 : Sotorasib chromatograms at A) Low-QC B) Medium-QC and C) High-QC level.

Effect of Matrix Constituents

Three duplicates of six blank plasma samples from six distinct groups were taken, spiked at low-QC and high-QC levels, and then compared with the same amounts in the other treatments. Low-QC samples had a CV% of 2.90 and High-QC samples had a CV% of 3.41, both of which are within 15% of the

Table 4: Sotorasib matrix effect outcomes

	LQC			*HQC*		
S.No	*Peak area in absence of matrix*	*Peak area in presence of matrix*	*Matrix factor*	*Peak area in absence of matrix*	*Peak area in presence of matrix*	*Matrix factor*
1	13754	13108	95.31	148364	141421	95.32
2	13692	13167	96.17	147590	151103	102.38
3	13703	13877	101.27	147952	142744	96.48
4	13792	14121	102.39	148035	150033	101.35
5	13688	13390	97.83	147894	152730	103.27
6	13781	13377	97.07	148954	144858	97.25
Mean			98.34			99.34167
± SD			2.85			3.39
%CV			2.90			3.41

Table 5: The stability data of sotorasib in human plasma under different storage conditions

	LQC		*MQC*		*HQC*	
Storage condition	*Accuracy (Mean%)*	*Precision (RSD%)*	*Accuracy (Mean%)*	*Precision (RSD%)*	*Accuracy (Mean%)*	*Precision (RSD%)*
Post preparative (10°C for 24 hours)	94.23	3.25	95.25	2.36	102.38	3.28
Short-term (at 25°C for 19 hours)	96.31	4.09	97.41	1.07	103.92	2.74
Long-term (at -70°C for 36 days)	102.38	1.92	103.21	1.99	97.84	2.64
Three freeze and thaw (3 cycles)	102.64	1.87	101.11	3.67	95.43	3.75
Stock solution (20 hours at 25°C)	94.95	3.24	96.28	4.08	102.64	1.92
Stock solution (17day at 2 ~ 8°C)	98.26	3.04	102.84	3.22	95.37	2.63

acceptance standards. The findings of the matrix factors are given in Table 4.

Application of Stability Studies

According to FDA regulations, stability tests were conducted across various environmental conditions. Three freeze/thaw cycles were performed, as well as short-term (at 25°C for 19 hours), long-term (at -70°C for 36 days), post-preparative (10°C for 24 hours), and stock solution stability conditions.[19] Results from stability experiments showed that plasma samples of sotorasib were stable throughout the chromatographic procedure, extraction, and storage under a variety of circumstances. Table 5 displays stability statistics.

Carry-over Effects

A blank sample was estimated using the maximum concentration of the calibration standards in 6 repeats to determine any potential carryover. After the HQC, the peak response of the blank sample should not be more than 20% of the drug response of the LLoQ or higher than 5% of the response of apalutamide. The carryover was acceptable as per the validation guidelines.

CONCLUSION

A linear, precise and specific LC-MSMS technique was developed for the quantification of sotorasib in biological matrices. Chromatographic resolution was achieved with ODS Zorbax (50 × 4.6 mm, 2.1 µ) C18 column and a mobile phase composition of methanol, 0.1% formic acid and ACN in the proportion of 50:15:35 with 0.5 mL/min flow of the mobile solvent system from the stationary column procedure was executed by monitoring the established ionic transitions of m/z- 561.09/316.84 for sotorasib and 478.09/451.08 for apalutamide internal standard in multiple reaction monitoring. The linear plot regression line was y = 0.0001x + 0.0011 and with correction coefficient (r2) of 0.9996. The %CV outcomes for matrix effect at low-QC and high-QC levels were 2.90 and 3.41%, respectively. The percentage average recoveries for sotorasib in high-QC (11.25 µg/mL), MQC (7.50 µg/mL) and low-QC (1.05 µg/mL) were 102.41, 98.07 and 102.41%, respectively. The obtained values were between 2.00 to 4.03% for the QC (0.375, 1.05, 7.50 and 11.25 µg/mL). The established technique was subjected for validation as per the FDA guiding principles and can be useful for evaluation of sotorasib in biological samples in quality control, forensic and bioavailability studies.

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