Brief Report

DOI: 10.5582/ddt.2023.01043

Development of a simple high-performance liquid chromatography-ultraviolet method for sotorasib quantification in human plasma: Implications for therapeutic drug monitoring

Eri Hikita[1,2], Yoshito Gando[1], Hideo Chubachi[3], Mikio Shirota[2,4], Akifumi Kushiyama[4,5], Takeo Yasu[1,2,4]

[1] Department of Medicinal Therapy Research, Pharmaceutical Education and Research Center, Meiji Pharmaceutical University, Tokyo, Japan;
[2] Department of Pharmacy, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan;
[3] Department of Pharmacy, Shonan-Fujisawa Tokushukai Hospital, Kanagawa, Japan;
[4] Bokutoh Hospital-Meiji Pharmaceutical University Joint Research Center, Tokyo, Japan;
[5] Department of Pharmacotherapy, Meiji Pharmaceutical University, Tokyo, Japan.

SUMMARY Sotorasib, an oral small-molecule inhibitor, reportedly exerts promising activity against Kirsten rat sarcoma viral oncogene homolog (*KRAS)*-mutant tumors. However, the currently administered dose may fail to represent the optimal dose based on the therapeutic efficacy. Herein, we developed a simple and sensitive method using high-performance liquid chromatography with ultraviolet (HPLC-UV) to measure the sotorasib concentration in human plasma. The sotorasib calibration curve exhibited linearity across the concentration range of 0.10-20.0 μg/mL (r^2 = 0.9999). The coefficients of intra- and inter-day validation ranged between 0.79-9.75% and 3.01-6.13%, respectively. The assay accuracy ranged between –3.14 and 5.18%, with > 98.5% recovery. Subsequently, we applied the developed method to estimate sotorasib concentrations in a patient with *KRAS* G12C-mutated non-small cell lung cancer. We anticipate that our HPLC-UV method will be valuable for assessing the safety and efficacy of sotorasib in larger patient cohorts.

Keywords HPLC, sotorasib, therapeutic drug monitoring, KRASG12C mutation, non-small cell lung cancer

1. Introduction

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene mutations have been frequently detected in patients with non-small cell lung cancer (NSCLC), colorectal cancer, pancreatic cancer, and other malignancies (*1,2*). Sotorasib is a small-molecule inhibitor that specifically inhibits *KRAS*. Preclinical studies have revealed that sotorasib can effectively suppress the phosphorylation of extracellular signal-regulated kinases, which serve as critical downstream effectors of *KRAS* (*3*). Consequently, sustained and complete tumor regression has been documented in murine models exhibiting *KRAS* G12C tumors (*4*). Importantly, sotorasib has been approved as the first targeted therapy for unresectable advanced or recurrent NSCLC with *KRAS* G12C mutation. However, treatment-related adverse events have necessitated dose modification (22.2%) and discontinuation of treatment (7.1%). The most common treatment-related adverse events that contribute to dose modification include diarrhea and elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood alkaline phosphatase (ALP) (*5*). Currently, sotorasib is administered at the maximum tolerated dose, as determined based on toxicity, although it does not necessarily reflect the optimal dose based on therapeutic efficacy (*6*).

Sotorasib is predominantly metabolized by cytochrome P450 (CYP)3A; hence, caution should be exerted when administering sotorasib concomitantly with CYP3A4 inducers and inhibitors. Furthermore, the absorption of sotorasib may be reduced when administered with antacids and proton pump inhibitors, potentially compromising treatment outcomes (*7*). Considering the pharmacokinetic profile, a daily dose of 960 mg sotorasib resulted in a coefficient of variation (CVs) of 98.3% for the maximum plasma concentration (C_{max}) (*8*), indicating substantial interindividual variability in blood concentrations. Therefore, careful consideration of blood sotorasib levels is warranted, emphasizing the importance of therapeutic drug monitoring (TDM) in

patients undergoing sotorasib therapy. The recommended sotorasib dose is 960 mg, with an initial dose reduction of 480 mg and a subsequent reduction of 240 mg suggested on encountering adverse reactions. However, comparable areas under the concentration-time curve from 0 h to 24 h ($AUC_{0–24h}$) and C_{max} values of sotorasib, ranging between 180 and 960 mg, have been documented following daily administration (*6*). During the dose escalation phase of a previous trial, low-dose sotorasib was found to afford overall response rates between 25 and 50% in patients with NSCLC, similar to the overall response rate achieved with 960 mg (*6*). Therefore, implementing sotorasib TDM to achieve a C_{max} equivalent to 960 mg may facilitate cost reduction in routine medical care.

To date, the sotorasib concentration in human plasma has only been measured using liquid chromatography-mass spectrometry (LC-MS/MS) (*9*). The LC-MS/MS system is expensive, and its introduction is limited to a few facilities. Conversely, high-performance liquid chromatography-ultraviolet (HPLC-UV) systems remain popular owing to their low initial cost and high utility in general hospitals. However, to the best of our knowledge, there have been no reports regarding the measurement of sotorasib concentrations in human plasma using HPLC-UV. Therefore, we developed a simple HPLC-UV method to estimate sotorasib concentrations in human plasma.

2. Methods

2.1. Instrumentation and reagents

The HPLC system consisted of a pump (PU-4180; Jasco, Tokyo, Japan), UV detector (UV-4075; Jasco), and autosampler (AS-4550; Jasco). Analysis was performed using an octadecylsilyl column (Capcell Pak C18 MG II; 250 mm × 4.6 mm; i.d., 5 μm; Osaka Soda, Osaka, Japan) equipped with a Capcell Pak C18 MG II guard column (10 mm × 4.0 mm; Osaka Soda). Sotorasib and bosutinib were acquired from Toronto Research Chemicals, Inc. (Ontario, Canada). The mobile phases comprised HPLC-grade acetonitrile, methanol, and water (Kanto Chemical Co., Inc., Tokyo, Japan) and KH_2PO_4 (Wako, Osaka, Japan).

2.2. Preparation of stock and working solutions

Stock solutions of sotorasib and bosutinib were prepared in acetonitrile and methanol, respectively, at a concentration of 1 mg/mL. The sotorasib stock solution was diluted in acetonitrile to obtain working solutions of 0.5, 1.25, 5.0, 12.5, 25.0, and 100.0 μg/mL. Bosutinib was diluted in methanol to obtain a 10 μg/mL working solution. Subsequently, the prepared stock and working solutions were stored at −80°C until use.

2.3. Assay procedure

A 10 μL working solution of sotorasib was vortexed with 50 μL of plasma in a 2.0 mL microtube (ClickFit 2.0 mL, Trerf Lab, Switzerland) for 10 s. Subsequently, 60 μL of sotorasib-spiked plasma, 10 μL of the internal standard (IS; 10 μg/mL bosutinib), and 130 μL of acetonitrile chilled to −20°C were added. The mixture was vortexed for 1 min and centrifuged at 15,000× *g* for 10 min at 4°C. The resulting supernatant (30 μL) was subjected to HPLC analysis. The detection wavelength was set to 230 nm, and the mobile phase comprised 0.5% KH_2PO_4 (pH 4.5) and acetonitrile (65:35, v/v) at a flow rate of 1.0 mL/min.

2.4. Calibration and validation

Calibration concentrations of sotorasib ranged from 0.10 to 20.0 μg/mL, and assay recovery and accuracy were determined at these concentrations. The assay precision was evaluated by analyzing five sets of control samples on the same day (intra-day) and on five different days (inter-day) at concentrations of 0.10, 0.25, 1.0, 2.5, 5.0, and 20.0 μg/mL.

2.5. Sample stability

Sotorasib stability in plasma samples was assessed at three different concentrations (0.10, 2.5, and 20.0 μg/mL). To establish benchtop stability, five samples (n = 5) stored at 25°C for 6 h were evaluated. Processed sample stability was evaluated by storing five samples (n = 5) at 4°C for 24 h. To determine long-term stability, five samples (n = 5) were stored at −60°C for four weeks and then assessed. Finally, freeze-and-thaw stability was determined by subjecting five samples (n = 5) to three cycles of freezing at −60°C and thawing.

2.6. Clinical application

Written informed consent was obtained from a patient with *KRAS* G12C-mutated NSCLC, and blood samples were collected for analysis. This case report measured the sotorasib blood levels of a single patient using our assay system. Samples were obtained at 2 h (C_{max}) after administering 960 mg of sotorasib on days 9, 13, 20, and 33. Plasma samples were obtained by centrifuging blood samples at 1,500× *g* for 15 min, which were subsequently stored at −80°C until analysis.

3. Results and Discussion

Figure 1a presents the chromatogram of the blank plasma sample, and Figure 1b shows the representative chromatogram of a plasma sample containing 0.10 μg/mL of sotorasib. The retention times for sotorasib and IS (bosutinib) were 14.5 and 10.8 min, respectively. The six-point sotorasib standard calibration curve exhibited linearity across the concentration range of 0.10-20.0 μg/

mL (r^2 = 0.9999). The lower limit of quantification for sotorasib was 0.10 μg/mL, with a recovery rate exceeding 95.8%. Considering this concentration range, the intra- and inter-day CVs ranged between 0.79-9.75% and 3.01-6.13%, respectively (Table 1). The assay accuracy ranged between –3.14-5.18%. The plasma stability of sotorasib was assessed under various conditions (Table 2). No significant sotorasib degradation was detected, and final concentrations were maintained within 91.43-108.05% of theoretical values.

In the present study, we developed a straightforward and highly sensitive HPLC-UV method to quantify plasma sotorasib concentrations in clinical settings. We believe this study makes a groundbreaking contribution to the management of patients receiving sotorasib therapy. The precision and accuracy of intra- and inter-assay variations and stability under diverse conditions adhered to the guidelines outlined by the Food and Drug Administration (*10*). Only one previous method for measuring sotorasib in human plasma samples has been reported, *i.e.*, an LC-MS/MS-based method, which requires a plasma volume of 20 μL for measurement (*9*). Conversely, the method developed in the present study allowed sotorasib quantification using minimal plasma volumes (as little as 10 μL); therefore, no further invasive procedures are required in addition to the usual blood collection.

Plasma concentrations of sotorasib were evaluated in samples collected from a male patient in his seventies, who was diagnosed with *KRAS* G12C-mutated NSCLC and undergoing second-line treatment with sotorasib. The patient had received sotorasib at a dose of 960 mg. Liver function tests revealed that the patient had AST, ALT, γ-glutamyl transpeptidase (γ-GTP), and ALP levels within the normal range prior to initiating sotorasib therapy. On day 33 following sotorasib administration, the patient exhibited an elevated γ-GTP level corresponding to grade 3, ALT level corresponding to grade 2, AST level corresponding to grade 1, and ALP level corresponding to grade 1, as per the Common Terminology Criteria for Adverse Events version 5.0. Consequently, sotorasib was discontinued on the following day. The plasma sotorasib concentrations on days 9, 13, 20, and 33 were 2.40 (Figure 1c), 4.55, 4.18, and 5.25 μg/mL, respectively. Figure 2 illustrates the trends in AST, ALT, γ-GTP, ALP, and C_{max} of sotorasib. The patient was concurrently taking vildagliptin, apixaban, hydrocortisone, metformin, risperidone, rosuvastatin, and yokukansan. Nine days after sotorasib discontinuation, elevated AST and ALT levels rapidly returned to normal.

In patients treated with sotorasib, the median (range) time to onset of grade 3 hepatotoxicity was found to be 9.1 (3.1-18.7) weeks (*11*). Considering our patient, the sudden onset of sotorasib-induced elevated γ-GTP, ALT, AST, and ALP levels was observed on day 33. Moreover, the C_{max} (5.25 μg/mL) of our patient on day 33 was similar to the C_{max} (5.39 μg/mL) previously reported for patients treated with 960 mg of sotorasib

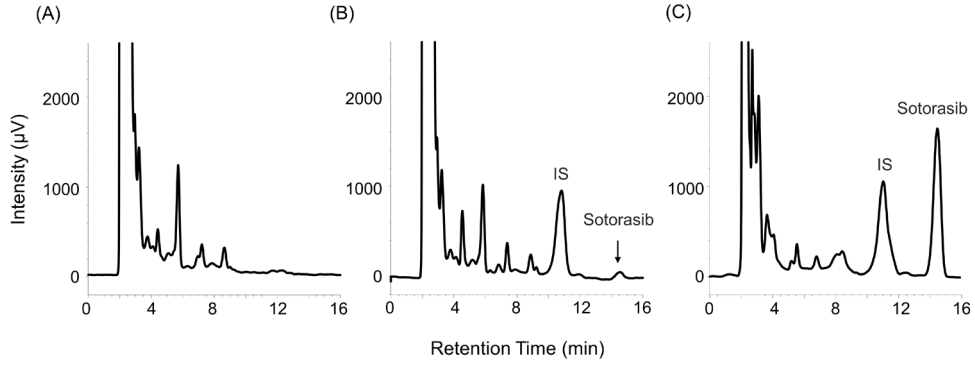

Figure 1. Chromatograms of (A) blank plasma sample, (B) plasma sample containing sotorasib 0.1 μg/mL, and (C) plasma sample from the patient receiving sotorasib 960 mg after 2 h on day 9 (2.40 μg/mL).

Table 1. Intra-day and inter-day accuracy and precision

Sotorasib added (μg/mL)	Intra-day (n = 5)			Inter-day (n = 5)			Recovery (%)
	Mean ± SD (μg/mL)	CV (%)	Accuracy (%)	Mean ± SD (μg/mL)	CV (%)	Accuracy (%)	
0.10	0.11 ± 0.01	9.75	5.18	0.10 ± 0.00	3.04	-0.68	95.83
0.25	0.25 ± 0.01	5.75	-1.91	0.25 ± 0.02	6.13	-0.43	106.92
1.0	0.97 ± 0.02	2.55	-2.79	0.99 ± 0.04	4.47	-1.42	101.18
2.5	2.45 ± 0.05	2.17	-2.17	2.48 ± 0.11	4.25	-0.71	100.84
5.0	4.95 ± 0.15	3.02	-0.98	4.98 ± 0.15	3.01	-0.39	101.51
20.0	19.22 ± 0.15	0.79	-3.88	19.37 ± 1.02	5.27	-3.14	102.32

CV, coefficient of variation; SD, standard deviation.

Table 2. Stability analyses under various conditions (n = 5)

Sotorasib added (μg/mL)	Stability condition (%)			
	Benchtop mean ± SD	Processed sample mean ± SD	Long-term 4 weeks mean ± SD	Freeze-and-thaw mean ± SD
0.10	108.05 ± 10.96	97.75 ± 6.14	100.76 ± 6.93	95.58 ± 4.60
2.5	96.26 ± 1.81	96.02 ± 2.07	92.27 ± 1.42	94.99 ± 4.19
20.0	91.43 ± 2.58	96.38 ± 2.25	91.90 ± 1.33	92.03 ± 2.96

SD, standard deviation.

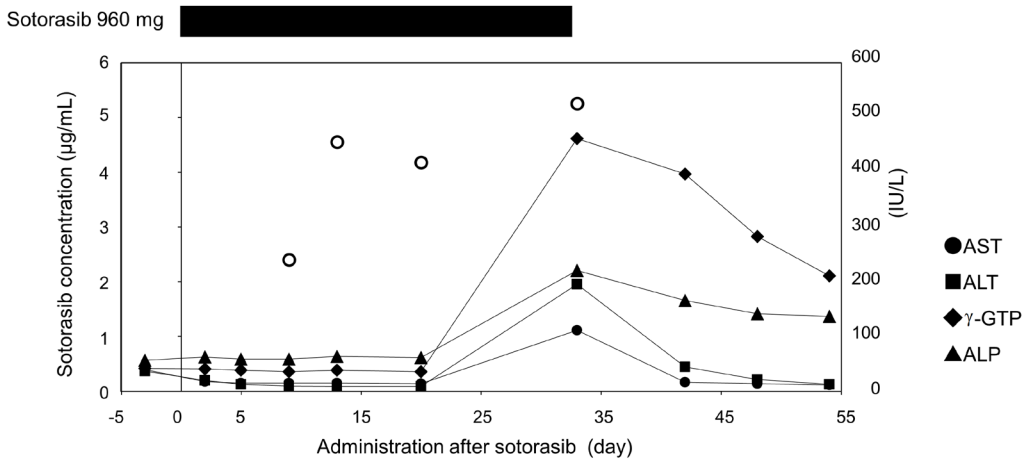

Figure 2. Trends for AST, ALT, γ-GTP, ALP, and the maximum concentration (C_{max}) of sotorasib. White circles indicate sotorasib C_{max}, black circles indicate AST, black squares indicate ALT, black diamonds indicate γ-GTP, and black triangles indicate ALP. AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

(*6*). Consequently, it is unlikely that the elevated levels of γ-GTP, ALT, AST, and ALP could be solely attributed to the high sotorasib C_{max}. No significant exposure-response relationships were identified for treatment-related adverse events (*12*). Conversely, the C_{max} value was 2.40 μg/mL on day 9 upon reaching a steady state, subsequently displaying an increasing trend to 4.55, 4.18, and 5.25 μg/mL after day 13. Moreover, elevated blood sotorasib concentrations have been reported in patients with hepatic dysfunction (*7*). Therefore, monitoring sotorasib C_{max} may facilitate the early detection of hepatotoxicity through changes in sotorasib C_{max} prior to the elevation of γ-GTP, ALT, AST, and ALP. Although our evaluation was limited to sotorasib C_{max} and GTP, ALT, AST, and ALP levels in a single patient, we plan to elucidate the relationship between altered sotorasib blood levels and hepatotoxicity in future studies using our developed HPLC-UV method.

Previous reports have failed to detect any correlation between the dose (180-960 mg) and steady-state drug exposure. Similarly, evidence suggesting a relationship between dose and response rate is lacking. Certain patients exhibit antitumor responses at lower than standard doses, suggesting that 960 mg could be deemed an excessive dose (*4*). Additionally, low-dose sotorasib may reduce gastrointestinal toxicity (*13*). Accordingly, TDM using the currently developed method to measure blood levels of sotorasib could help reduce the sotorasib dose and related gastrointestinal toxicity.

Nevertheless, the limitations of the present study should be considered. Firstly, we evaluated samples from only one patient. Consequently, it remains unknown whether sotorasib concentrations can be accurately measured in patients taking medications other than the seven drugs administered by the aforementioned patient. In future studies, we intend to employ the developed method to measure sotorasib in a larger cohort of patients to establish the accuracy of our sotorasib measurements.

Sotorasib has been assessed in clinical trials for pancreatic and colorectal cancer (*14,15*) and could be expanded to other indications in the future. Our method could be valuable for assessing the safety and efficacy of sotorasib in treating diverse cancers.

In conclusion, we developed a simple and sensitive HPLC-UV-based method for determining the concentration of sotorasib in a clinical setting. Further investigation utilizing our assay is warranted to elucidate the relationship between sotorasib blood levels and clinical efficacy, as well as adverse effects.

Funding: This research was funded by the Japan Society for the Promotion of Science (JSPS) KAKENHI [grant number JP20K16095].

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Román M, Baraibar I, López I, Nadal E, Rolfo C, Vicent S, Gil-Bazo I. *KRAS* oncogene in non-small cell lung cancer: Clinical perspectives on the treatment of an old target. Mol Cancer. 2018; 17:33.
2. Uras IZ, Moll HP, Casanova E. Targeting *KRAS* mutant non-small-cell lung cancer: Past, present and future. Int J Mol Sci. 2020; 21:4325.
3. Lito P, Solomon M, Li LS, Hansen R, Rosen N. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. Science. 2016; 351:604-608.
4. Canon J, Rex K, Saiki AY, *et al*. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature. 2019; 575:217-223.
5. Skoulidis F, Li BT, Dy GK, *et al*. Sotorasib for lung cancers with *KRAS* p.G12C mutation. N Engl J Med. 2021; 384:2371-2381.
6. Nakajima EC, Drezner N, Li X, *et al*. FDA approval summary: Sotorasib for *KRAS G12C*-mutated metastatic NSCLC. Clin Cancer Res. 2022; 28:1482-1486.
7. U.S. Food and Drug Administration, Drugs@FDA [database on the internet]. Sotorasib USPI. *https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/214665s000lbl.pdf* (accessed June 1, 2023).
8. Hong DS, Fakih MG, Strickler JH, *et al*. $KRAS^{G12C}$ inhibition with sotorasib in advanced solid tumors. N Engl J Med. 2020; 383:1207-1217.
9. Wong P, Akrami A, Houk B, Vuu I, James CA. Validation of an LC-MS/MS method for the determination of sotorasib, a $KRAS^{G12C}$ inhibitor, in human plasma. Bioanalysis. 2022; 14:1281-1292.
10. US DHHS, FDA, CDER, CVM. Guidance for industry: Bioanalytical method validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research and Center for Veterinary Medicine, Rockville, MD, 2018.
11. Dy GK, Govindan R, Velcheti V, *et al*. Long-term outcomes and molecular correlates of sotorasib efficacy in patients with pretreated *KRAS* G12C-mutated non-small-cell lung cancer: 2-year analysis of CodeBreaK 100. J Clin Oncol. 2023; 41:3311-3317.
12. CDER application #214665Orig1s000 sotorasib. 2020. *https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/214665Orig1s000MultidisciplineR.pdf* (accessed June 1, 2023).
13. Ratain MJ, Tannock IF, Lichter AS. Dose optimization of sotorasib: Is the US Food and Drug Administration sending a message? J Clin Oncol. 2021; 39:3423-3426.
14. Strickler JH, Satake H, George TJ, *et al*. Sotorasib in *KRAS* p.G12C-mutated advanced pancreatic cancer. N Engl J Med. 2023; 388:33-43.
15. Fakih MG, Kopetz S, Kuboki Y, *et al*. Sotorasib for previously treated colorectal cancers with $KRAS^{G12C}$ mutation (CodeBreaK100): A prespecified analysis of a single-arm, phase 2 trial. Lancet Oncol. 2022; 23:115-124.

Received June 29, 2023; Revised August 2, 2023; Accepted August 12, 2023.

**Address correspondence to:*
Takeo Yasu, Department of Medicinal Therapy Research, Pharmaceutical Education and Research Center, Meiji Pharmaceutical University; 2-522-1, Noshio, Kiyose, Tokyo 204-8588, Japan.
E-mail: yasutakeo-tky@umin.ac.jp

Released online in J-STAGE as advance publication August 19, 2023.