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Short communication

Determination of salirasib (*S-trans*,*trans*-farnesylthiosalicylic acid) in human plasma using liquid chromatography–tandem mass spectrometry

Ming Zhao, Ping He, Linping Xu, Manuel Hidalgo, Dan Laheru, Michelle A. Rudek*

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

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ABSTRACT

A liquid chromatography/tandem mass spectrometric (LC/MS/MS) assay was developed for the quantitative determination of salirasib (*S-trans*,*trans*-farnesylthiosalicylic acid, FTS) in human plasma. Sample pretreatment involved liquid–liquid extraction with methyl *t*-butyl ether of 0.5-mL aliquots of lithium heparin plasma spiked with the internal standard, *S-trans*,*trans*-5-fluoro-farnesylthiosalicylic acid (5-F-FTS). Separation was achieved on Waters X-Terra^{TM} C_{18} (50 mm × 2.1 mm i.d., 3.5 μm) at room temperature using isocratic elution with acetonitrile/10 mM ammonium acetate buffer mobile phase (80:20, v/v) containing 0.1% formic acid at a flow rate of 0.20 mL/min. Detection was performed using electrospray MS/MS by monitoring the ion transitions from *m/z* 357.2 → 153.0 (salirasib) and *m/z* 375.1 → 138.8 (5-F-FTS). Calibration curves were linear in the concentration range of 1–1000 ng/mL. A 5000 ng/mL sample that was diluted 1:10 (v/v) with plasma was accurately quantitated. The values for both within day and between day precision and accuracy were well within the generally accepted criteria for analytical method (<8.0%). This assay was subsequently used for the determination of salirasib concentrations in plasma of cancer patients after oral administration of salirasib at a dose of 400 mg.

1. Introduction

Salirasib (*S-trans*,*trans*-farnesylthiosalicylic acid, FTS) is an orally bioavailable small molecule, with demonstrated activity as a Ras-antagonist [1,2]. Activated RAS proteins are involved in tumor formation and maintenance [3]. Farnesyltransferase inhibitors (FTIs) inhibit Ras farnesylation and can inhibit RAS transforming activity [4]. Two Ras isoforms, K-Ras and N-Ras, can undergo an alternative prenylation pathway to become activated [5,6]. Salirasib interferes with Ras-binding and thus can effectively dislodge all Ras isoforms (H-Ras, K-Ras, and N-Ras) [7,8]. Antitumor activity has been demonstrated in a variety of preclinical models [9–12]. Due to its novel mechanism of action and preclinical antitumor activity, salirasib is in clinical development.

To comprehensively characterize the clinical pharmacokinetic (PK) profile of salirasib, a specific, reproducible and accurate method for the quantitation of salirasib was necessary. A previously published LC/MS/MS method quantitated salirasib from 3 to 1000 ng/mL in mouse plasma using a 0.1 mL aliquot with a run time of 5.2 min [10]. Here, we describe an analytical method for determination of salirasib concentrations in human plasma based on LC/MS/MS method with electrospray negative ionization and using a single step liquid–liquid extraction with methyl *tert*-butyl ether.

2. Experimental

2.1. Chemical and reagents

Salirasib and *S-trans*,*trans*-5-fluoro-farnesylthiosalicylic acid (5-F-FTS) were a gift from Concordia Pharmaceuticals, Inc. (Ft Lauderdale, FL, USA). Ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals were HPLC grade and obtained from EM Science (Gibbstown, NJ, USA). Deionized water was obtained from a Milli-Q-UF system (Millipore, Milford, MA, USA) and used throughout in all aqueous solutions. Drug-free (blank) human plasma originated from Pittsburgh Blood Plasma, Inc. (Pittsburgh, PA, USA).

2.2. Stock solutions, calibration standards, and quality control samples

Stock solutions of salirasib at a concentration of 1 mg/mL were prepared in duplicate in methanol and stored in glass vials at −20 °C. The area counts for each of the duplicated aliquots were

* Corresponding author at: The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Bunting-Blaustein Cancer Research Building, Room 1M87, 1650 Orleans Street, Baltimore, MD 21231-1000, USA. Tel.: +1 410 614 6321; fax: +1 410 502 0895.
E-mail address: mrudek2@jhmi.edu (M.A. Rudek).

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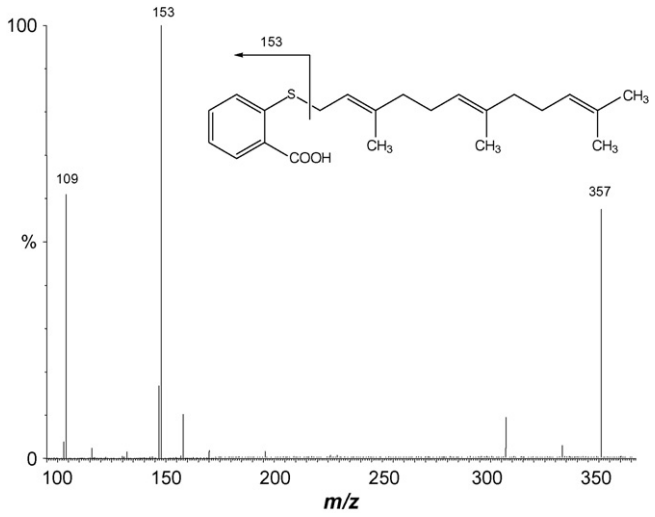

Fig. 1. Daughter scan mass spectrum of salirasib with monitoring at *m/z* 357.2 → 153.0.

checked in quintuplicate, and if the mean value for area counts was within 5%, the stock solutions were then stored in a glass vial at −20 °C. Stock solutions were diluted in acetonitrile–water (1:1, v/v) on each day of analysis to spike into pooled human plasma to prepare a calibration curve standards and quality control (QC) samples. Seven calibration samples containing salirasib were prepared at the concentrations: 1, 5, 10, 50, 100, 500, and 1000 ng/mL. Quality control samples were prepared fresh daily and independently at four different concentrations: 1 [lower limit of quantitation (LLOQ)], 3, 80, and 800 ng/mL. An additional dilution QC was prepared at 5000 ng/mL and diluted 1:10 (v/v) in pooled human plasma for quantitation. For long-term stability, QC samples were prepared as a batch and stored at −70 °C.

2.3. Sample preparation

Prior to extraction, frozen plasma samples were thawed in a water bath at ambient temperature. After mixing on a vortex-mixer, a 0.5 mL aliquot of plasma was added to a screw-cap glass tube (16 mm × 125 mm) containing 5 mL of methyl *tert*-butyl ether and 5F-FTS (20 ng/mL), which was used as the internal standard. The tube was capped and mixed vigorously for 10 min on automated multi-tube shaker, followed by centrifugation at 2000 × *g* for 10 min at ambient temperature. The top organic layer was transferred to a disposable borosilicate glass culture tube (13 mm × 100 mm) and evaporated to dryness at 30 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of acetonitrile/water (50:50, v/v) by vortex mixing (30 s) and ultrasonication (5 min). The sample was transferred to a 250-μL polypropylene autosampler vial, sealed with a Teflon crimp cap, and a volume of 50 μL was injected onto the HPLC instrument for quantitative analysis using a temperature-controlled autosampling device operating at 10 °C.

2.4. Chromatographic and mass-spectroscopic conditions

Chromatographic analysis was performed using a Waters Model 2690 separations system (Milford, MA, USA). Separation of the analyte from potentially interfering material was achieved at ambient temperature using Waters X-Terra C_{18} column (50 mm × 2.1 mm i.d., 3.5-μm), protected by a RP18 guard column (10 mm × 2.1 mm i.d., 3.5-μm; Milford, MA, USA). The mobile phase used for the chromatographic separation was composed of acetonitrile containing 0.1% formic acid/10 mM ammonium acetate (pH 6.3) (80:20, v/v), and was delivered isocratically at a flow rate of 0.2 mL/min. The total run time was 5 min. The column effluent was monitored using a Micromass Quattro LC triple-quadrupole mass-spectrometric detector (Beverly, MA, USA). The instrument was equipped with an electrospray interface, and controlled by the Masslynx version 3.5 software (Micromass). The samples were analyzed using an electrospray probe in negative ionization mode operating at a cone voltage of 30 V for both salirasib and 5-F-FTS. Samples were introduced into the interface through a heated nebulized probe (350 °C). The spectrometer was programmed to allow the $[MH]^-$ ion of salirasib at *m/z* 357.2 and that of 5-F-FTS at *m/z* 375.1 to pass through the first quadrupole (Q1) and into the collision cell (Q2). The collision energy was set at 15 eV for salirasib and internal standard. The daughter ions for salirasib (*m/z* 153.0) and 5-F-FTS (*m/z* 171.2) were monitored through the third quadrupole (Q3) (Fig. 1). Argon was used as collision gas at a pressure of 0.0027 mbar, and the dwell time per channel was 0.5 s for data collection.

2.5. Calibration curves

Calibration curves for salirasib were computed using the ratio of the peak area of analyte to internal standard by using a least-squares linear-regression analysis. The parameters of each calibration curve were used to compute back-calculated concentrations of calibration standards and to obtain values for the QC samples and unknown samples by interpolation.

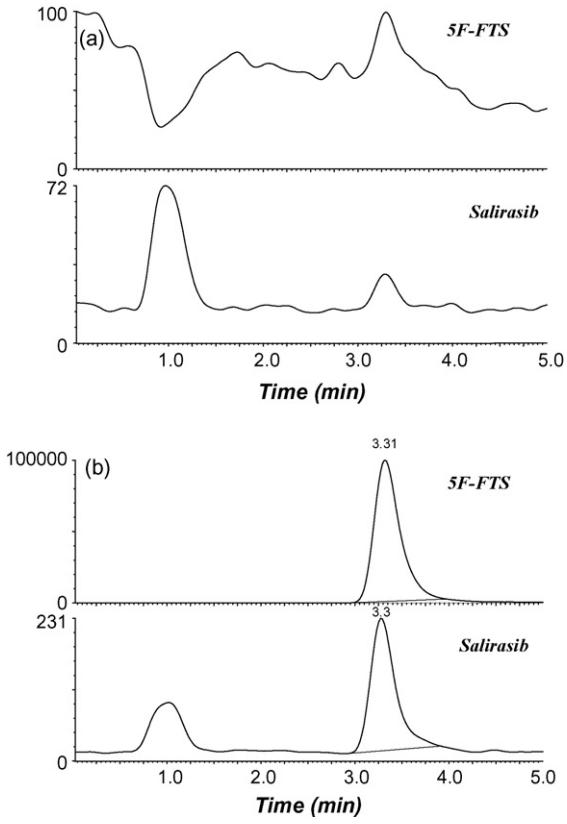

Fig. 2. Selected ion chromatogram of blank plasma (a) and plasma spiked with salirasib (1 ng/mL) (b). The following mass-to-charge (*m/z*) ratios were monitored 357.2 → 153.0 for salirasib and 375.1 → 171.2 for 5F-FTS. The retention times for salirasib and 5F-FTS were both 3.3 min.

2.6. Method validation

Method validation runs were performed on four consecutive days, and included a calibration curve processed in duplicate, QC samples in quintuplicate, and a single plasma blank and zero-level standard (blank with internal standard). The accuracy and precision of the assay were assessed by the mean relative percentage deviation from the nominal concentrations and the within-run and between-run precision, respectively. Estimates of the between-run precision were obtained by one-way analysis of variance (ANOVA) as previously described [13]. The specificity of the method, extraction efficiency, and select stability tests (stock solution and autosampler) of salirasib were assessed.

2.7. Patient samples

The samples analyzed were from one cancer patient enrolled to a clinical trial where salirasib was administered orally at a dose of 400 mg twice daily for 3 weeks every 4 weeks in combination with gemcitabine given once weekly for 3 weeks. Blood samples were collected in tubes containing lithium heparin at baseline (pretreatment) and up to 24 h following a single dose of salirasib on day 7 (at steady-state). Blood samples were maintained at room temperature and centrifuged at 2000 × *g* for 10 min within 30 min of collection. The resultant plasma was stored at −70 °C until analysis. The clinical protocol was approved by the local institutional review board and all patients provided written informed consent before entering the study.

3. Results and discussion

3.1. Detection and chromatography

The mass spectrum of salirasib showed protonated molecular ions ([MH$^-$]) at *m/z* 357.2. One of the major fragments observed was at *m/z* 153.0, which was selected for subsequent monitoring in the third quadrupole (Fig. 1). The mass spectrum of the internal standard, 5F-FTS, showed a [MH$^-$] at *m/z* 375.1, and the high collision energy gave one major product ion at *m/z* 171.2 (data not shown).

No peaks were observed in the chromatograms of blank plasma from 19 donors when monitored for salirasib. Additionally, carryover was not observed in either blank plasma or zero-level standard (blank with internal standard) after injection of the high quality control (data not shown). Representative chromatograms of plasma spiked with 5F-FTS and salirasib are shown in Fig. 2. The mean (±S.D.) retention times for salirasib and 5F-FTS were 3.28 ± 0.10 and 3.32 ± 0.12 min, respectively, with an overall chromatographic run time of 5 min.

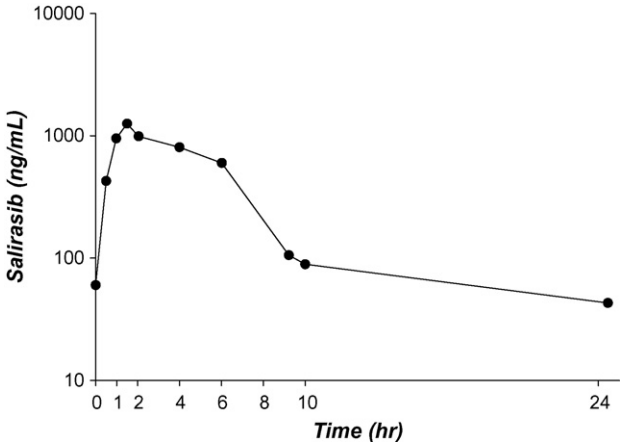

Fig. 3. Plasma concentration–time profile in a cancer patient after 13 doses of salirasib (400 mg) administered orally.

3.2. Linearity of detector responses

The calculated peak area ratios of salirasib to 5F-FTS versus the nominal concentration of the analyte displayed a linear relationship in the tested range of 1–1000 ng/mL using a weighting factor of 1/*x*. A mean least-squares linear-regression correlation coefficient of great than 0.99 was obtained in all analytical runs. For each point on the calibration curves for salirasib, the concentrations back-calculated from the equation of the regression analysis were always within 4.5% of the nominal value. The within-run and between-run variability (precision), expressed as the percentage relative standard deviation, was less than 10.2% (data not shown). The LLOQ for salirasib was established at 1 ng/mL. At this concentration, the mean (±S.D.) signal-to-noise ratio was 47.1 ± 24.9 and the accuracy was 101.4%.

3.3. Accuracy, precision, and recovery

For QC samples prepared by spiking human plasma with salirasib, the within-run and between-run variability (precision), expressed as the percentage relative standard deviation, was less than 8.0% (Table 1). The extraction efficiency of salirasib was concentration independent and larger than 73.8%. The extraction efficiency of 5F-FTS was 5.2%.

3.4. Analyte stability

Salirasib stock solution was very stable in methanol at room temperature with no degradation found in 6 h (data not shown). Long-term stability studies for 196 days demonstrate salirasib is

Table 1
Assessment of accuracy, precision, recovery, and stability

Nominal concentration (ng/mL)	Accuracy[a] (%)	Precision[a] (%)		Recovery (%)	Stability (% of initial)	
		Within-run	Between-run		Autosampler stability[b]	Long-term stability (−70 °C)[c]
1	98.0	8.0	5.7	–[d]	–[d]	–[d]
3	99.9	2.8	3.5	86.5	97.3	111.1
80	99.8	1.9	1.2	73.8	–[d]	–[d]
800	104.5	1.2	1.7	83.4	102.8	105.3
500[e]	104.4	2.4	4.4	–[d]	–[d]	95.4

[a] Performed in quintuplicate on four separate days.
[b] Performed repeatedly for 12.4 h with one sample that was extracted from plasma.
[c] Stability data were expressed as mean percentage of salirasib concentration (performed in triplicate) determined at 196 days relative to that at time zero (%).
[d] Not done.
[e] Sample diluted 1:10 with plasma prior to extraction.

stable at −70 °C. Salirasib was stable up to 13 h on the autosampler without any significant degradation, allowing for more than 149 samples to be analyzed simultaneously within a single chromatographic run. Stability studies for a similar analytical method were conducted for Concordia Pharmaceuticals by Ricerca Biosciences, LLC (Concord, OH) [14]. Plasma spiked with salirasib stored at room temperature for up to 6 h indicated that salirasib was stable during this time period. QC samples prepared in human plasma undergoing three freeze–thaw cycles showed no significant degradation for salirasib.

3.5. Plasma concentration–time profile

The present LC/MS/MS method was successfully applied to study the pharmacokinetics of salirasib in a cancer patient receiving 400 mg salirasib orally. Fig. 3 shows a salirasib plasma concentration–time profile after multiple doses. The maximum plasma concentration achieved was 1255 ng/mL, which occurred at 1.5 h, and salirasib was detectable for 24 h.

4. Conclusion

In conclusion, we have developed and validated an assay for measuring salirasib in human plasma. This method will be used to characterize the clinical pharmacology of salirasib in combination therapy in cancer patients to further optimize salirasib treatment schedules for future clinical evaluation.

Disclosure

This work was supported by Concordia Pharmaceuticals, Inc. Concordia Pharmaceuticals is a client of Averion International Corp. Dr. Rudek also is a paid consultant to Averion International. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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