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Short communication

A liquid chromatography/tandem mass spectrometry assay to quantitate MS-275 in human plasma☆

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Abstract

A rapid, sensitive and selective method was developed and validated using LC/MS/MS for determination of MS-275 in human plasma. Sample preparation involved a single step liquid–liquid extraction by the addition of 0.2 ml of plasma with 5 ml acetonitrile/*n*-butyl-chloride. Separation of the compounds of interest, including the internal standard paclitaxel, was achieved on a Waters X-Terra™ C_{18} (50 mm × 2.1 mm i.d., 3.5 μm) analytical column using a mobile phase consisting of acetonitrile/ammonium acetate (pH 2.9; 2 mM)(60:40, v/v) containing 0.1% formic acid and isocratic flow at 0.15 ml/min for 3 min. The analytes were monitored by tandem-mass spectrometry with electrospray positive ionization. Linear calibration curves were generated over the range of 0.5–100 ng/ml with values for the coefficient of determination of >0.99. The values for both within day and between day precision and accuracy were well within the generally accepted criteria for analytical methods (<15%). This method was subsequently used to measure concentrations of MS-275 in cancer patients receiving an oral weekly dose of 4 mg/m^2.
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Keywords: MS-275; LC/MS/MS; Pharmacokinetics

1. Introduction

MS-275 (3-pyridylmethyl-*N*-{4-[(2-amino-pyenyl)-carbamoyl]-benzyl}-carbamate (Fig. 1)) is an orally active bioavailable histone deacetylase inhibitor [1]. In preclinical studies, MS-275 has demonstrated antitumor activity against a variety of human cancer cell lines [1,2]. Antitumor activity and a lack of toxicity has been observed following oral MS-275 administration in human tumor xenograft models [1,3]. MS-275 was safe and achieved concentrations associated with preclinical efficacy in phase I trials of MS-275 alone [4,5]. MS-275 is currently being evaluated in Phase I clinical trials in patients with refractory solid tumors with the drug being administered once weekly in combination with 13-cis-retinoic acid at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins [6].

To comprehensively characterize the clinical pharmacology of MS-275, a selective, reproducible and accurate method for the quantitation of MS-275 was necessary. In recent years, only one analytical method based on liquid chromatography single quadrupole mass spectrometry assay has been reported [7]. The lowest limitation of quantitation (LLOQ) was 1 ng/ml utilizing a 0.1 ml aliquot with a total run time of 8 min. Here, we describe an analytical method for the determination of MS-275 that is 2 times more sensitive and approximately three times faster than the previously reported analytical method.

2. Experimental

2.1. Chemical and reagents

MS-275 was kindly supplied by the Developmental Therapeutics Program, Cancer Therapy Evaluation Program, National Institute of Health (Bethesda, MD, USA). The internal standard, paclitaxel, was purchased from Sigma Co. (St. Louis, MO,

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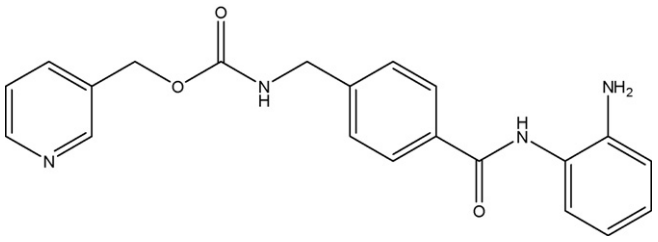

Fig. 1. Chemical structure of MS-275.

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 from healthy donors originated from Pittsburgh Blood Plasma, Inc. (Pittsburgh, PA, USA).

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

Stock solutions of MS-275 at a concentration of 1 mg/ml were prepared in duplicate in methanol and stored in glass vials at −20 °C. The stock solutions were diluted in blank human plasma on each day of analysis to prepare 6 calibration standards containing MS-275 for human plasma samples at the following concentrations: 0.5, 1, 5, 10, 50 and 100 ng/ml. Quality control (QC) samples were prepared fresh daily and independently at four different concentrations for MS-275 including: 0.5 (lower limit of quantitation, LLOQ), 1.5, 8, and 80 ng/ml. An additional dilutional QC was prepared at 800 ng/ml and diluted 1:10 (v/v) in pooled human plasma for quantitation. For long-term and freeze-thaw stability, QC samples were prepared as a batch and stored at −20 °C.

A 1.0 mM stock solution of paclitaxel was prepared in methanol and stored in glass vials at −20 °C. A 20 μl aliquot of the 1.0 mM internal standard stock solution was added into 1 l of acetonitrile/n-butylchlorde (1:4, v/v) for a final concentration of 20 nM at the time of analysis for plasma samples.

2.3. Sample preparation

Prior to extraction, frozen samples were thawed in a water bath at ambient temperature. A 0.2 ml aliquot of plasma was added to a screw cap culture test tube (16 mm × 125 mm) containing 5 ml of acetonitrile/*n*-butylchlorde (1:4, v/v) solution and paclitaxel (20 nM), which was used as the internal standard. The tube was mixed vigorously for 30 s on a vortex-mixer, followed by centrifugation at 1200 × *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 40 °C under gentle stream of nitrogen. The residue was reconstituted in 100 μl 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 20 μl was injected onto the HPLC instrument using a temperature-controlled autosampling device operating at 10 °C.

2.4. Chromatographic and mass-spectroscopic conditions

Chromatographic analysis was performed using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA). Separation of the analytes 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 (3.5 μm; Milford, MA, USA). The mobile phase used for the chromatographic separation was composed of acetonitrile/ammonium acetate (pH 2.9; 2 mM)(70:30, v/v) containing 0.1% formic acid, and was delivered isocratically at a flow rate of 0.2 ml/min. The column effluent was monitored using an API 3000 triple-quadrupole mass-spectrometric detector (Applied Biosystems, Foster City, CA, USA). The instrument was equipped with an electrospray interface, operated in a positive mode and controlled by the Analyst version 1.2 software (Applied Biosystems). The spectrometer was programmed to allow the $[MH^+]$ ion of MS-275 at *m/z* 377.2 and that of the internal standard at *m/z* 854.5 to pass through the first quadrupole (Q1) and into the collision cell (Q2). The daughter ions for MS-275 (*m/z* 104.0) and the internal standard (*m/z* 105.0) were monitored through the third quadrupole (Q3).

2.5. Calibration curves

Calibration curves for MS-275 were computed using the ratio of the peak area of analyte and internal standard by using a least-squares linear regression analysis. The parameters of each calibration curve were used to compute back-calculated concentrations and to obtain values for the QC samples and unknown samples by interpolation.

2.6. Method validation

Method validation runs were performed on four consecutive days and included a calibration curve processed in duplicate and QC samples in quintuplicate. The accuracy and precision of the assay was 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 [8]. The specificity, extraction efficiency, and stability of MS-275 were assessed.

2.7. Patient samples

The samples analyzed were from one cancer patient enrolled to a clinical trial where MS-275 was administered at a dose of 4 mg/m^2 once weekly for 3 weeks every 4 weeks in combination with 13-cis-retinoic acid given twice daily. Blood samples were collected in heparinized tubes at baseline (pre-treatment) and up to 8 days following administration of the first dose of MS-275. Blood samples were maintained at room temperature and centrifuged at 1000 × *g* for 10 min within 30 min of collection.

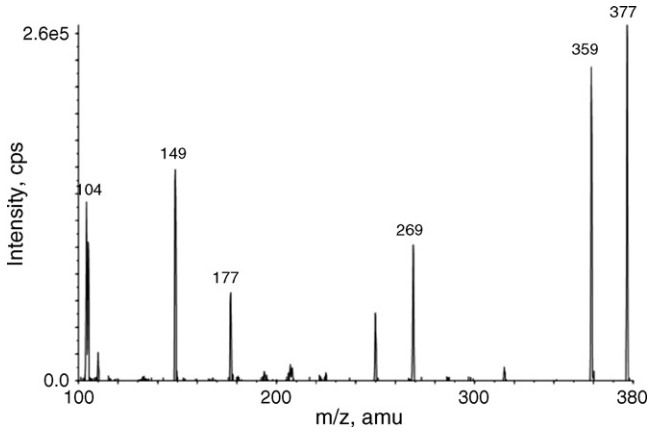

Fig. 2. Daughter mass spectrum of MS-275 with monitoring at *m/z* 377.2 → 104.0.

The resultant plasma was stored at −20 °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 [6].

3. Results and discussion

3.1. Detection and chromatography

The mass spectrum of MS-275 showed a protonated molecular ion ([MH$^+$]) at *m/z* 377.2. One of the major fragments observed was at *m/z* 104.0, which was selected for subsequent monitoring in the third quadrupole (Fig. 2). The mass spectrum of the internal standard, paclitaxel, showed a [MH$^+$] at *m/z* 854.5, and the high collision energy gave one major product ion at *m/z* 105.0 (data not shown).

No peaks were observed in the chromatograms of blank plasma from 6 donors when monitored for MS-275. Representative chromatograms of plasma spiked with internal standard and MS-275 are shown in Fig. 3. The mean (±standard deviation) retention times for MS-275 and paclitaxel under the optimal conditions were 0.95 ± 0.02 and 1.35 ± 0.02 min with an overall chromatographic run time of 3 min.

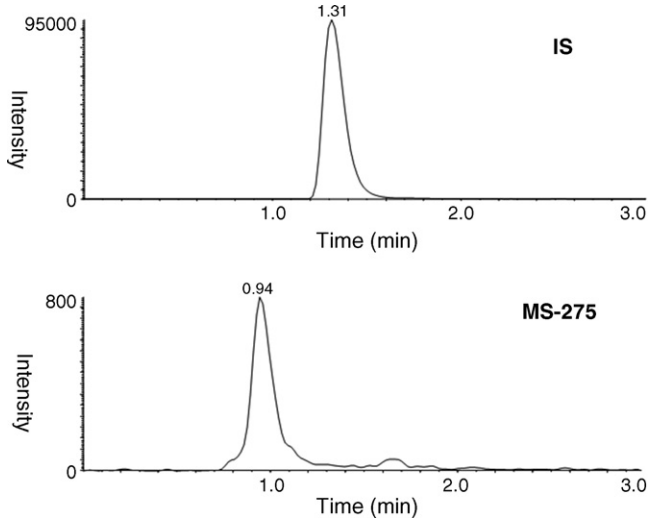

Fig. 3. Selected ion chromatogram of plasma spiked at the LLOQ concentration of MS-275 (0.5 ng/ml) containing the internal standard at 20 nM. The retention times for MS-275 and internal standard were approximately 0.95 ± 0.02 and 1.32 ± 0.02 min.

3.2. Linearity of detector responses

The calculated peak area ratios of MS-275 to paclitaxel versus the nominal concentration of the analyte displayed a linear relationship in the tested range of 0.5–100 ng/ml using a weighting factor of 1/*x*. A mean least-squares linear-regression correlation coefficient of greater than 0.99 was obtained in all analytical runs. For each point on the calibration curves for MS-275, the concentrations back-calculated from the equation of the regression analysis were always within 5.3% of the nominal value.

The LLOQ for MS-275 was established at 0.5 ng/ml for human plasma. At this concentration, the mean (±standard deviation) signal-to-noise ratio was 81.6 ± 35.1 and the accuracy and precision less than 20% from a total of 20 observations.

3.3. Accuracy, precision, and recovery

For QC samples prepared by spiking human plasma with MS-275, the within-run and between-run variability (precision), expressed as the percentage relative standard deviations, was less than 15%, except the LLOQ was 16.5%. Likewise, the mean predicted concentration (accuracy) was less than 8.1% of the nominal value (Table 1). The relative recovery of MS-275 was greater than 71% at all QC concentrations when extracted with paclitaxel. The absolute recovery for paclitaxel was 78% (data not shown).

3.4. Analyte stability

QC samples prepared in human plasma undergoing three freeze-thaw cycles showed no significant degradation (<6.9%) for MS-275. Long-term stability studies from 294 days demonstrate MS-275 is stable at −20 °C. MS-275 was stable up to 4 h on the autosampler without any significant degradation, allowing

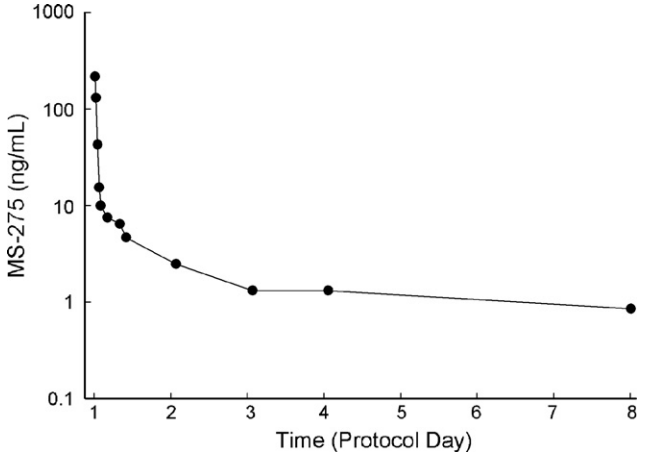

Fig. 4. MS-275 plasma concentration-time profile on week 1 in a patient receiving an oral weekly dose of 4 mg/m^2.

Table 1
Assessment of accuracy, precision, and recovery

Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)		Recovery (%)	No. of Samples
		Within-run	Between-run		
Human plasma[a]					
0.5	108.0	16.5	7.1	–[b]	20
1.5	105.1	11.4	–[c]	83.8	20
8	105.0	6.0	2.0	71.1	20
80	108.1	7.5	2.7	86.0	20
800 (1:10)[d]	106.1	11.7	3.2	–[b]	12

[a] Performed in quintuplicate on four separate days.
[b] Not done.
[c] No significant variation was observed as a result of performing the assay in different runs.
[d] Sample diluted 1:10 (v/v) prior to analysis and performed in triplicate on four separate days.

for more than 80 samples to be analyzed simultaneously within a single chromatographic run.

3.5. Plasma concentration–time profiles

The present LC–MS–MS method was successfully applied to study the pharmacokinetics of MS-275 in a cancer patient receiving oral MS-275 as a weekly dose of 4 mg/m^2 for 3 weeks every 4 weeks in combination with 13-cis-retinoic acid. Fig. 4 shows a MS-275 plasma concentration-time profile on week 1. Following a single oral dose of MS-275 4 mg/m^2, the maximum plasma concentration achieved was 216 ng/ml, which occurred at 0.25 h, and MS-275 was detectable through 8 days.

4. Conclusion

In conclusion, we have developed and validated an assay for measuring MS-275 in human plasma. This method is being used to characterize the clinical pharmacology of MS-275 in combination therapy in cancer patients to further optimize MS-275 treatment schedules for future clinical evaluation.

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