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Determination of BMS-284756, a new quinolone, in mouse serum by high-performance liquid chromatography with fluorescence detection

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Abstract

A sensitive, simple, and accurate method for determination of BMS-284756, a novel des-F(6)-quinolone antimicrobial agent in mouse serum was developed by HPLC with fluorescence detection. Sample preparations were carried out by protein precipitation with the addition of acetonitrile, followed by evaporation of the acetonitrile to dryness. The resultant residual was then reconstituted in 0.01 *M* HCl and injected onto a Nucleosil 100 10 μm, C_{18} 25 cm×4.6 mm analytical column. The mobile phase consisted of acetonitrile–0.01 *M* NaH_2PO_4 (20:80, v/v) with 0.01 *M* tetrabutylammonium hydrogen sulfate. The fluorescence of the column effluent was monitored at an excitation wavelength of 290 nm and an emission wavelength of 418 nm. The assay was shown to be linear from 0.2 to 10.0 μg/ml (R^2=0.998). Mean recovery was determined as 95.1%. Inter- and intra-assay precisions were <6% RSD. The HPLC method developed has been applied to determine the pharmacokinetics of BMS-284756 in a murine bacterial infection model.

Keywords: BMS-284756; Quinolones

1. Introduction

BMS-284756 {1-cyclopropyl-8-(difluoromethoxy)-7-[(1*R*)-(1-methyl-2,3-dihydro-1H-5-isoindolyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid methanesulfonate monohydrate} (Fig. 1), is a novel des-F(6)-quinolone antibacterial agent currently under development. It has broad-spectrum antibacterial activity against Gram-positive and Gram-negative organisms [1–3]. Currently, no report is available regarding high-performance liquid chromatographic (HPLC) assay of BMS-284756 in biological matrices. In order to support preclinical pharmaco-

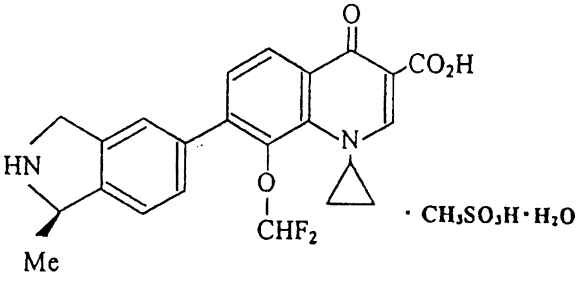

Fig. 1. Chemical structure of BMS-284756.

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kinetic/pharmacodynamic studies requiring the quantitation of BMS-284756, we developed a sensitive, simple, and accurate method for the quantitative determination of BMS-284756 in mouse serum.

2. Experimental

2.1. Materials

BMS-284756 research reference standard [lot No. 807T-3(A), 78.7% purity] was obtained from Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, USA. Moxifloxacin, used as internal standard (lot No. 502610, 87.9% purity) was obtained from Bayer, Pharmaceutical Division, West Haven, CT, USA. All the solvents and reagents were for HPLC-use grade from Sigma, St. Louis, MO, USA. The water was filtered through the Millipore Milli-Q UV-Plus system (18 MΩ-cm; Waters, Milford, MA, USA). The blank mouse serum was obtained from healthy ICR mice (Harlan Sprague Dawley, Indianapolis, IN, USA).

2.2. Preparation of standard stock solution

The stock solution of BMS-284756 was prepared by dissolving 25.41 mg of BMS-284756 in 10 ml Milli-Q UV-Plus water in a glass flask to obtain a nominal concentration of 2 mg/ml.

2.3. Preparation of internal standard stock solution

The stock solution of internal standard was prepared by dissolving 11.4 mg of moxifloxacin in 10 ml Milli-Q UV-Plus water to obtain a nominal concentration of 1 mg/ml.

2.4. Preparation of assay standard samples

BMS-284756 standard samples (0.2, 1.0, 3.0, 5.0, 6.0, and 10.0 μg/ml) were prepared by spiking control mouse serum with appropriate volumes of the stock solution prepared mentioned above. Quality control (QC) samples (0.5, 4.0, and 8.0 μg/ml) were independently prepared in the same manner.

2.5. Extraction procedure

A 200-μl aliquot of standard, QC, or unknown mouse serum sample and 50 μl internal standard were placed into labeled 1.7-ml snap-cap tubes. A 500-μl volume of acetonitrile was added to each tube and mixed thoroughly by vortexing for 30 s, followed by centrifugation at 3600 *g* for 6 min. The supernatants were transferred to labeled clean tubes and were placed under a stream of nitrogen at 40°C to remove acetonitrile. The residuals were reconstituted in 200 μl 0.01 *M* HCl with vortexing for 30 s, and centrifugation for 6 min. The solution was then transferred to WISP vials for injection onto the HPLC column (Alltech Nucleosil 100 C_{18}, 10 μm, 25 cm×4.6 mm).

2.6. Assay conditions

Extracted samples were loaded onto a WISP autosampler (WISP 717 plus; Waters). The mobile phase consisted of acetonitrile–0.01 *M* NaH_2PO_4 (20:80, v/v) with 0.01 *M* tetrabutylammonium hydrogen sulfate was delivered by a Waters HPLC pump (Model 515) in isocratic mode at a flow-rate of 1.3 ml/min. The fluorescence of the column effluent was monitored by a fluorescence detector (Model 980; Applied Biosystems, Foster City, CA, USA) at an excitation wavelength of 290 nm and an emission wavelength of 418 nm. A chromatography data system (EZChrome Elite; Scientific Software, San Ramon, CA, USA) was used for data acquisition. The retention times for BMC-284756 and I.S. were 12.4 and 6.0 min, respectively.

2.7. Validation procedure

2.7.1. Specificity

The degree of interference by endogenous serum constituents with BMS-284756 and the internal standard was assessed by inspection of chromatograms derived from processed blank and spiked serum samples used to define the assay lower limit of

quantitation (LLQ), and also from processed blank samples injected during each analytical run.

2.7.2. Range of reliable response

The analytical standards were processed and analyzed in duplicate. The processed samples were injected onto the HPLC column to determine if the detector output was a linear function of concentration over the nominal concentration range of the standards (0.2–10 μg/ml). The linear regression of the curve for peak height ratio versus concentration was weighted by $1/x$ (the reciprocal of the nominal BMS-284756 concentration). Linearity and reproducibility were determined by the slope, intercept, and coefficient of determination (R^2) values.

2.7.3. Lower limit of quantitation

LLQ samples (0.2 μg/ml) were prepared with pooled control mouse serum. Six LLQ and control samples were analyzed with a standard curve. The accuracy of individual LLQ samples was determined as the percentage of deviation (%DEV) of the observed concentration from the nominal concentration. The precision for LLQ replicate samples was determined by the percentage of relative standard deviation (RSD).

2.7.4. Accuracy and precision

Quality control samples (0.5, 4.0, and 8.0 μg/ml) were analyzed to evaluate accuracy and precision of the assay. Six replicate QC samples at each concentration were analyzed with standards (in duplicate) in a single sequence for the intra-assay assessment. For inter-assay assessment QC samples at each concentration were analyzed in duplicate with standards on six subsequent occasions. The accuracy of the assay was determined by calculating the %DEV of the predicted concentrations from the nominal concentrations. The precision of the assay was determined by calculating the RSD.

2.7.5. Recovery

Recovery of BMS-284756 from process samples was evaluated by comparing a standard curve prepared in the usual manner with a standard curve spiked directly into 0.5 *M* phosphate buffer, pH 6.8 (neat standard curve), to produce the expected nominal concentrations. The percent recovery of BMS-284756 was calculated using the formula:

$$\%\ \text{Recovery} = \frac{\text{Slope of processed standard curve}}{\text{Slope of neat standard curve}} \cdot 100$$

The percent recovery of the internal standard was calculated using the formula:

$$\%\ \text{Recovery} = \frac{\text{Mean response of processed peak}}{\text{Mean response of neat peak}} \cdot 100$$

2.7.6. Stability

The freeze–thaw stability of BMS-284756 in mouse serum was assessed over three freeze–thaw cycles. QC samples in duplicate at concentrations of 0.5, 4.0, and 8.0 μg/ml were frozen at −70°C, and thawed at room temperature three consecutive times, then the samples were processed and analyzed to determine the BMS-284756 concentration. The data were analyzed by comparing the measured concentrations to the respective nominal values.

The stability of extracted BMS-284756 on the autosampler was evaluated by analyzing representative extracted mouse samples reconstituted in 0.01 *M* HCl (QC samples 8.0 μg/ml) at intervals over a 48 h period. The data were evaluated by using the peak height responses of BMS-284756, the peak height responses of the internal standard, and the peak height ratios for the first injections (0 h), as the respective nominal values, and calculating the %DEV from this mean values for all the individual values.

2.8. Pharmacokinetics

The assay was applied to the study of pharmacokinetic/pharmacodynamic profile of BMS-284756 against *Streptococcus pneumoniae* in a neutropenic mouse model of thigh infection. Animals were administered a single oral dose of BMS-284756 at 25, 50, and 100 mg/kg. Blood samples were collected from six animals at specific sampling points. Samples were stored at −70°C until analysis.

3. Results and discussion

3.1. Specificity

Typical chromatograms obtained from processed blank and spiked serum samples are presented in Fig. 2. A good separation of BMS-284756 and its internal standard was obtained. No interfering peaks were found at the retention time of BMS-284756 and the internal standard.

3.2. Range of reliable response

Representative standard curve data for BMS-284756 in mouse serum are shown in Table 1. The observed peak height ratios of BMS-284756 to the

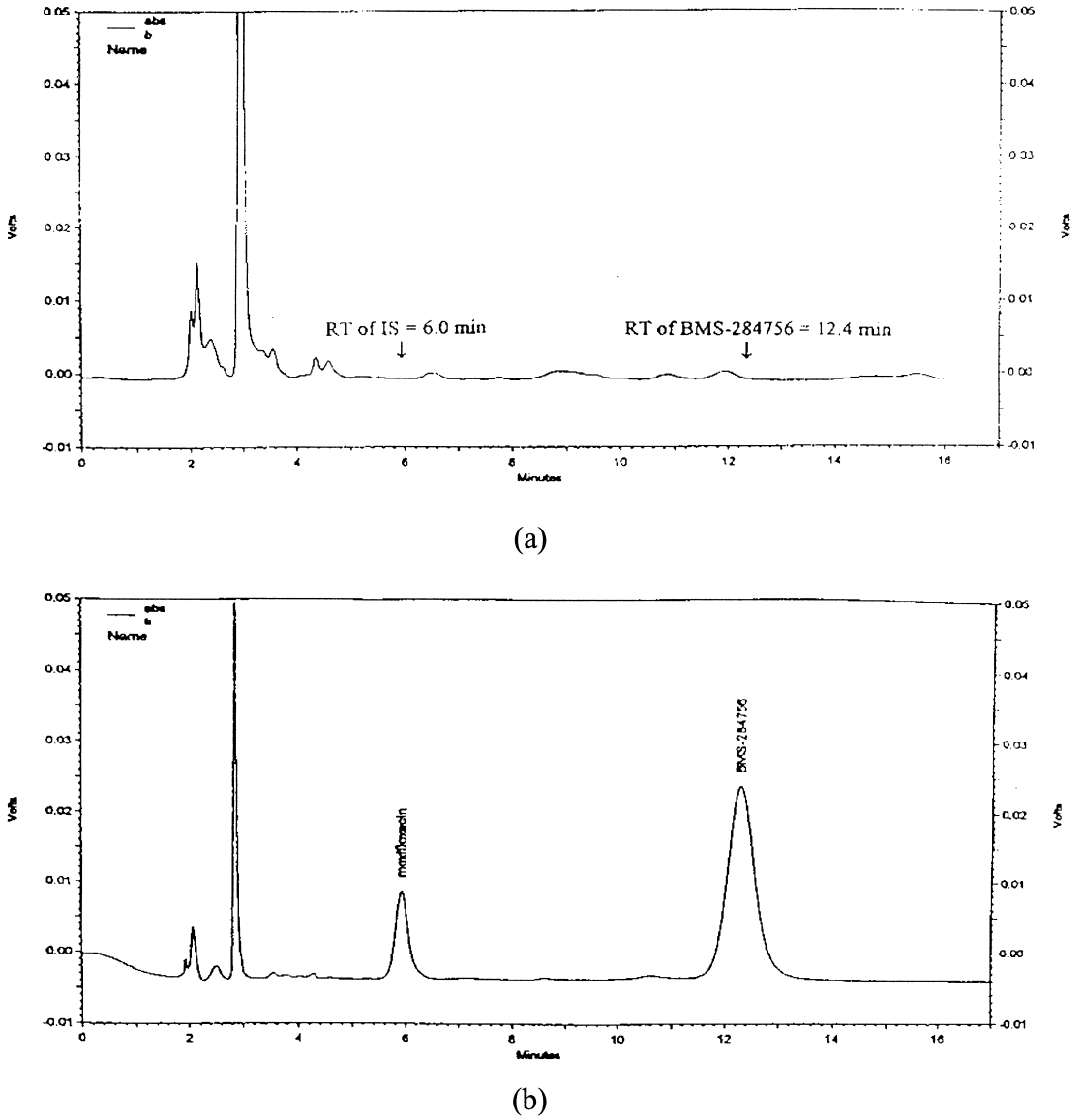

Fig. 2. Chromatograms of (a) extracted pooled blank mouse serum and (b) a mixture of BMS-284756 (8 μg/ml) and moxifloxacin (internal standard, 0.5 μg/ml) in mouse serum. Conditions as described in the Experimental section.

Table 1
Standard curve data of BMS-284756 in mouse serum

Normal concentration (μg/ml)	Weight	Response value	Mean response	RSD (%)	Predicted concentration (μg/ml)	%DEV	Mean concentration (μg/ml)	Mean %DEV
0.2	5.00	0.100	0.101	1.4	0.197	−1.5	0.200	0.0
0.2	5.00	0.102			0.204	2.0		
1.0	1.00	0.341	0.337	1.9	0.977	−2.3	0.963	−3.7
1.0	1.00	0.332			0.948	−5.2		
3.0	0.33	1.021	1.003	2.5	3.178	5.9	3.120	4.0
3.0	0.33	0.985			3.061	2.0		
5.0	0.20	1.584	1.567	1.5	5.000	0.0	4.947	−1.1
5.0	0.20	1.551			4.893	−2.1		
6.0	0.17	1.923	1.915	0.6	6.097	1.6	6.073	1.2
6.0	0.17	1.908			6.049	0.8		
10.0	0.10	3.115	3.094	0.9	9.955	−0.5	9.889	−1.1
10.0	0.10	3.074			9.822	−1.8		

Model: Peak height ratio = concentration × slope + intercept. $R^2 = 0.9998$.

internal standard were found to be linear over the concentration range of 0.2 to 10 μg/ml. The R^2 values for the standard curves were ≥0.998.

3.3. Lower limit of quantitation

The results of accuracy and precision evaluation for mouse serum LLQ samples indicate that all predicted concentration values were within ±10% of the nominal values. The mean accuracy as determined by %DEV was 8.3%, and the corresponding precision expressed as RSD was 2.38%.

3.4. Inter-assay accuracy and precision

The results of inter-assay accuracy and precision evaluation for QC samples are shown in Table 2. The inter-assay accuracy estimates for QC samples were 0.67, 4.92 and 0.10 %DEV for the QC samples of 0.5, 4.0, and 8.0 μg/ml, respectively. The corresponding precision estimates were 5.71, 2.99, and 3.53% RSD, respectively.

3.5. Intra-assay accuracy and precision

Table 3 contains the intra-assay accuracy and precision results for QC samples. The intra-assay accuracy estimates for QC samples were −7.33, 4.21, and 0.02 %DEV for the QC samples of 0.5,

Table 2
Inter-assay accuracy and precision for BMS-284756 in mouse serum

Run No.	Concentration (μg/ml)		
	0.50	4.00	8.00
1	0.50	4.25	7.56
2	0.52	4.32	7.96
3	0.48	4.32	8.34
4	0.46	4.17	8.01
5	0.53	4.00	7.90
6	0.53	4.12	8.28
Mean observed concentration	0.50	4.20	8.01
%DEV	0.67	4.92	0.10
RSD (%)	5.71	2.99	3.53

Table 3
Intra-assay accuracy and precision for BMS-284756 in mouse serum

Replication No.	Concentration (μg/ml)		
	0.50	4.00	8.00
1	0.46	4.14	7.92
2	0.46	4.21	7.55
3	0.49	4.16	7.94
4	0.46	4.19	8.06
5	0.46	4.15	8.26
6	0.45	4.16	8.28
Mean observed concentration	0.46	4.17	8.00
%DEV	−7.33	4.21	0.02
RSD (%)	2.95	0.63	3.36

4.0, and 8.0 μg/ml, respectively. The corresponding precision estimates were 2.95, 0.63, and 3.36% RSD, respectively.

3.6. Recovery

The recovery of BMS-284756 from the process serum standard curve samples was assessed by comparing to the aqueous standard curve. The extraction efficiency of the assay method, as determined by the ratio of the slopes of processed serum versus the unprocessed aqueous samples of BMS-284756 was 95.1%. For the internal standard, the corresponding recovery value, calculated by comparing the mean peak response of the processed internal standard against the neat internal standard, was 100.1% (data not shown).

3.7. Stability

Freeze–thaw stability of BMS-284756 was evaluated using the aforementioned method. Mean predicted concentrations after three freeze–thaw cycles were 0.49, 4.17, and 7.81 μg/ml for QCs with nominal concentrations of 0.5, 4.0, and 8.0 μg/ml, respectively. The corresponding %DEVs were −2.0, 4.3, and −2.4, respectively, and the RSDs were 1.5, 5.1, and 9.6%, respectively. Overall results indicate that BMS-284756 is stable in mouse serum samples following three freeze–thaw cycles.

The results of the stability of evaluation for BMS-284756 in extracted mouse serum at room temperature in the autosampler indicate that over a 32-h period, BMS-284756 was stable in extracted mouse serum reconstituted in 0.01 *M* HCl, noted by the %DEV of <10 compared to the nominal value. The stability of the internal standard in extracted mouse serum was also evaluated. The results indicate that the internal standard was stable for at least 32 h at room temperature in extracted mouse serum, reconstituted in 0.01 *M* HCl.

3.8. Pharmacokinetics

A typical serum concentration–time curve for BMS-284756 (50 mg/kg) from mice with pneumococci thigh infection is shown in Fig. 3. The concentrations obtained from all the dosages were pooled and analyzed with population approach (NONMEM, NONMEM Project Group, University of California at San Francisco, San Francisco, CA, USA) using a one-compartment model with first-order absorption and elimination. The parameter estimates for elimination half-life ($T_{1/2}$) and total clearance (CL/F) were 6.0 h and 0.808 l/h, respectively. The pharmacokinetic results of BMS-284756 in mice were further employed in designing the pharmacodynamic study of BMS-284756 in mouse model of pneumococci thigh infection and the subsequent analysis of the pharmacokinetic pharmacodynamic relationship of this compound.

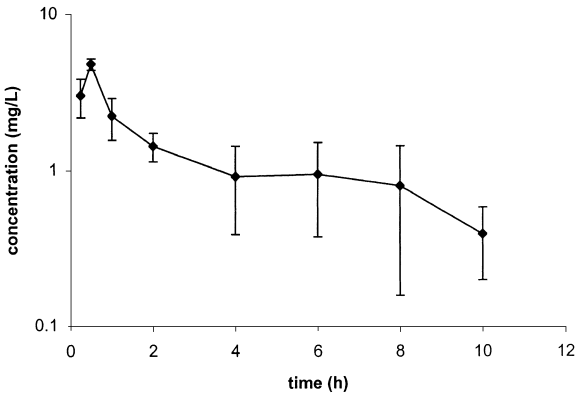

Fig. 3. Serum concentration–time profile for BMS-284756 following a 50 mg/kg oral dose in mice with pneumococci thigh infection.

4. Conclusions

The assay developed for the quantitation of BMS-284756 in mouse serum is sufficiently specific, accurate, sensitive, and reproducible. The inter- and intra-assay variability of QCs was less than 6% RSD, and the mean value of all QCs deviated from the nominal concentration by less than ±8%. Recovery evaluations showed that BMS-284756 was recovered from mouse serum at the rate of 95.1%. BMS-284756 in mouse serum was stable over three freeze–thaw cycles, and the extracted serum samples reconstituted in 0.01 *M* HCl were stable in the autosampler for at least 32 h. This assay can be used

for the preclinical pharmacokinetic / pharmacodynamic studies of requiring the quantitation of BMS-284756.

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