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A rugged and accurate liquid chromatography–tandem mass spectrometry method for quantitative determination of BMS-790052 in plasma

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ABSTRACT

To support toxicokinetic assessments, a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the quantification of BMS-790052 in rat, dog, monkey, rabbit and mouse K_2EDTA plasma. The drug was isolated from buffered samples using ISOLUTE C8 96-well solid phase extraction (SPE) plates. Chromatographic separation was achieved on a Waters Atlantis dC18 analytical column (2.1 mm × 50 mm, 5 μm) with detection accomplished using an API 4000 tandem mass spectrometer in positive ion electrospray and multiple reaction monitoring (MRM) mode. The standard curves, which ranged from 5.00 to 2000 ng/mL for BMS-790052, were fitted to a $1/x^2$ weighted linear regression model. The intra-assay precision (%CV) and inter-assay precision (%CV) were within 8.5%, and the assay accuracy (%Dev) was within ±7.1 for rat, dog, monkey, rabbit and mouse K_2EDTA plasma. This accurate, precise, and selective SPE/LC–MS/MS method has been successfully applied to analyze several thousands of non-clinical study samples.

1. Introduction

BMS-790052 (Fig. 1) is a novel first-in-class, highly potent and selective inhibitor of hepatitis C virus (HCV) non-structural protein 5A (NS5A), a multifunctional protein with key functions in HCV replication and modulation of cellular signaling pathways and the interferon response. BMS-790052 is the most potent HCV replication inhibitor described to date with 50% effective concentration (EC50) values in cell-based replicon assays of 9 and 50 pM against genotypes 1b and 1a, respectively [1]. Current interferon-based treatments for HCV are only effective in some populations [2–4] and are often associated with side effects.

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) using triple-quadrupole mass spectrometers with an atmospheric pressure ionization (API) source and operated under multiple reaction monitoring (MRM) mode has emerged as an enabling technology for quantitative bioanalysis in drug discovery and development due to its selectivity, specificity and sensitivity [5,6]. A ballistic gradient using a short, narrow-bore, reversed-phase column and a relatively high flow rate has become the preferred choice for bioanalysis, and the injection-to-injection cycle time has been shortened to less than five minutes while maintaining chromatographic resolution [7,8]. In our method development, we used a streamlined and efficient strategy for LC column and mobile phase screening followed by sample extraction screening (using solid phase extraction and liquid–liquid extraction) [9]. The selected method was optimized to reduce bioanalytical risks associated with phospholipids (matrix effects), and interference with potential metabolites.

In this manuscript, we report a validated LC–MS/MS method for the quantification of BMS-790052 in rat, dog, monkey, rabbit and mouse plasma, which have been used to support pre-clinical toxicokinetic studies for the investigational new drug (IND) application and post-IND studies. The method validation process was fully compliant with regulatory guidance under Good Laboratory Practices [10] and internal standard practice procedures (SOPs). This method utilized a stable-isotope labeled $^{13}C_{10}$-BMS-790052 as internal standard and solid phase extraction (SPE) to clean up plasma samples, which ensured good assay accuracy, precision and reproducibility. A full validation was first conducted in rat plasma followed by partial validations in dog, monkey, rabbit and mouse plasma.

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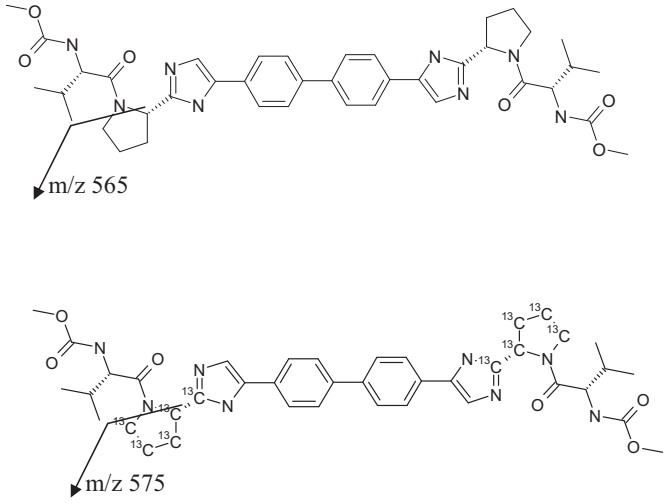

Fig. 1. Chemical structures of BMS-790052 (top) and its internal standard, $^{13}C_{10}$-BMS-790052 (bottom).

2. Materials and methods

2.1. Materials

BMS-790052 and the internal standard ($^{13}C_{10}$-BMS-790052) were provided by Bristol-Myers Squibb, Fig. 1. Acetonitrile, methanol, formic acid, acetic acid, isopropanol, ammonium acetate were the highest grades available. Drug-free rat, dog, monkey, rabbit, and mouse K_2EDTA plasma were purchased from Bioreclamation Inc. (Hicksville, NY, USA). The HPLC analytical columns (Atlantis dC18, 2.1 mm × 50 mm, 5 μm) were purchased from Waters Corporation (Milford, MA, USA). 96-well SPE plates (ISOLUTE C8, 25 mg) were from International Sorbent Technology (Mid Glamorgan, UK). The HPLC system was from Shimadzu Scientific Instruments Inc. (Kyoto, Japan) consisting of two LC-10AD VP pumps, a DGU-14A degasser, and a SIL-HTC autosampler. The mass spectrometer was a Sciex API 4000 from Applied Biosystems (Foster City, CA, USA). The Quadra 96 liquid handling robotic system was from Tomtec (Hamden, CT, USA).

2.2. Solution preparation

Solutions for SPE conditioning (50 mM ammonium acetate with 0.7% acetic acid), washing (50% acetonitrile in water), and eluting (0.1% formic acid in 47.5% methanol, 47.5% acetonitrile and 5% water) were prepared and stored at room temperature. HPLC mobile phases A (5.0 mM ammonium acetate with 0.01% acetic acid) and B (acetonitrile), a reconstitution solution (50% acetonitrile in the mobile phase A), and an autosampler wash solution (40% methanol and 40% isopropanol in water) were prepared and stored at room temperature. Stock solutions of BMS-790052 and the internal standard were prepared, respectively, at 1.00 mg/mL in methanol and stored at ~4 °C. An internal standard working solution (100 ng/mL) was prepared by diluting the stock solution with 30% methanol in 50 mM ammonium acetate with 0.7% acetic acid and stored at ~4 °C.

2.3. Calibration standards and quality control (QC) sample preparation

A standard working solution of 40.0 μg/mL was prepared by appropriate dilution of the 1.00 mg/mL BMS-790052 stock solution with methanol. The calibration standards at the concentrations of 5.00, 10.0, 20.0, 50.0, 100, 500, 1000, and 2000 ng/mL were prepared by spiking the standard working solution (40.0 μg/mL) into pooled drug-free K_2EDTA plasma followed by serial dilutions with plasma. The calibration standards were freshly prepared and used on the day of preparation. A separate QC stock solution (1.00 mg/mL) was prepared from a separate weighing. QC samples at the concentrations of 5.00 (Lower Limit of Quantitation QC, i.e., LLOQ QC), 15.0 (Low QC), 125 (Geometric Mean QC, i.e., GM QC), 1000 (Mid QC), 1600 (High QC), and 100000 (Dilution QC) ng/mL, were prepared from the stock solution followed by serial dilutions with a different lot plasma than used for the calibration standards. Aliquots of all QCs were placed into polypropylene tubes and stored at approximately −20 °C.

2.4. Solid phase extraction

Fifty-μL volume of plasma samples, blanks, calibration standards and QCs were pipetted into 96-well plates (Dilution QC was diluted 200-fold with drug-free plasma before pipetting into plates for the extraction). Internal standard working solution (50 μL of a 100 ng/mL solution) was added to each well and the mixture was vortexed for ~1 min, except that 50 μL of a solution of 30% methanol in 50 mM ammonium acetate with 0.7% acetic acid was added to double blank samples. A 200-μL volume of SPE conditioning solution was added to each sample and the mixture was vortexed for ~1 min. ISOLUTE C8 96-well extraction plates were pre-conditioned with 250 μL of methanol followed by 350 μL of SPE conditioning solution, and then loaded with the sample mixtures. The plates were sequentially washed with 200 μL of the SPE conditioning solution and 250 μL of the SPE washing solution, with an application of vacuum for 15–20 s after each addition. Finally, the analyte was eluted slowly from the plates by 2 × 200 μL of the SPE elution solution to a deep-well collection plate. The eluant was evaporated for ~30 min at ~40 °C under nitrogen, and then reconstituted in 100 μL of the SPE reconstitution solution.

2.5. LC–MS/MS

The HPLC column was operated at room temperature under a gradient program with mobile phases A and B at a total flow-rate of 0.4 mL/min: 40%B for 0.2 min, 40–65%B for 1.5 min, 65–95%B for 0.1 min, 95%B for 1.0 min, 95–40%B for 0.1 min, and 40%B for 1.0 min. The autosampler was washed with isopropanol/methanol/water (40:40:20, v/v/v) after each injection. The reconstituted samples were kept at 5 °C in the autosampler. Five μL of the reconstituted samples were injected into LC–MS/MS and analyzed under positive electrospray MRM mode (m/z 739 > 565 for BMS-790052, and m/z 749 > 575 for $^{13}C_{10}$-BMS-790052, Fig. 2) with the mass spectrometer parameters of Collision Gas (6 units), Curtain Gas (30 units), Ion Source Gas 1 (30 units), Ion Source Gas 2 (60 units), TurboIonSpray Voltage (4500 V), Turbo Probe Temperature (500 °C), Dwell Time (200 ms), Declustering Potential (130 V), Entrance Potential (10 V), Collision Energy (59 eV), and Collision Cell Exit Potential (16 V).

3. Results and discussion

3.1. Analytical method development

We used a streamlined method screening and optimization strategy [9] during method development. The HPLC conditions (mobile phases and columns) and the sample extraction method were chosen following the screening processes as illustrated in Fig. 3. In step 1, the volatile salts (ammonium bicarbonate, ammonium acetate, ammonium formate, and ammonium carbonate), acids (formic acid and acetic acid), and organic solvents (acetonitrile, methanol, and a mixture of acetonitrile/isopropanol) were