Journal of Chromatography B, 879 (2011) 2064–2072

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

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

Hao Jiang[a], Jianing Zeng[a,*], Yuzhong Deng[a,1], Yuan-Qin Xia[b], Zheng Ouyang[b], Mohammed Jemal[b], Theodora W. Salcedo[c], Mark E. Arnold[a]

[a] Bioanalytical Sciences Department, Bristol-Myers Squibb Company, Princeton, NJ 08540, USA
[b] Bioanalytical Research Department, Bristol-Myers Squibb Company, Princeton, NJ 08540, USA
[c] Immunotoxicology Department, Bristol-Myers Squibb Company, New Brunswick, NJ 08901, USA

ARTICLE INFO

Article history:
Received 1 April 2011
Accepted 23 May 2011
Available online 30 May 2011

Keywords:
LC–MS/MS
Quantitative
BMS-790052
Plasma
HCV

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.

* Corresponding author.
E-mail address: jianing.zeng@bms.com (J. Zeng).
[1] Current address: Drug Metabolism and Pharmacokinetics, Genentech, South San Francisco, CA 94080, USA.

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.05.036

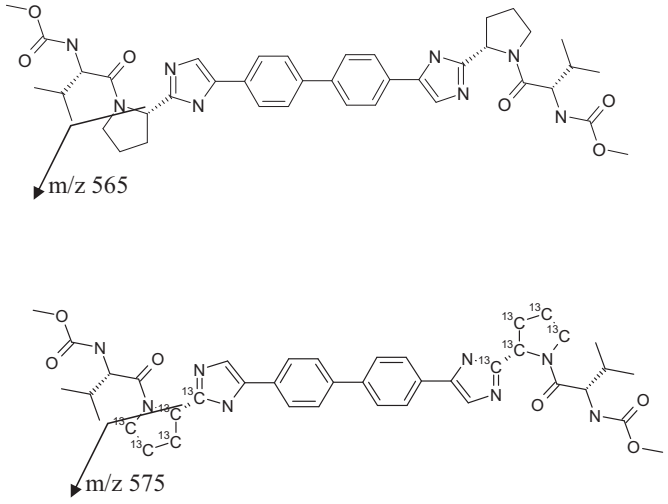

Fig. 1. Chemical structures of BMS-790052 (top) and its internal standard, $^{13}C_{10}$-BMS-790052 (bottom).

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

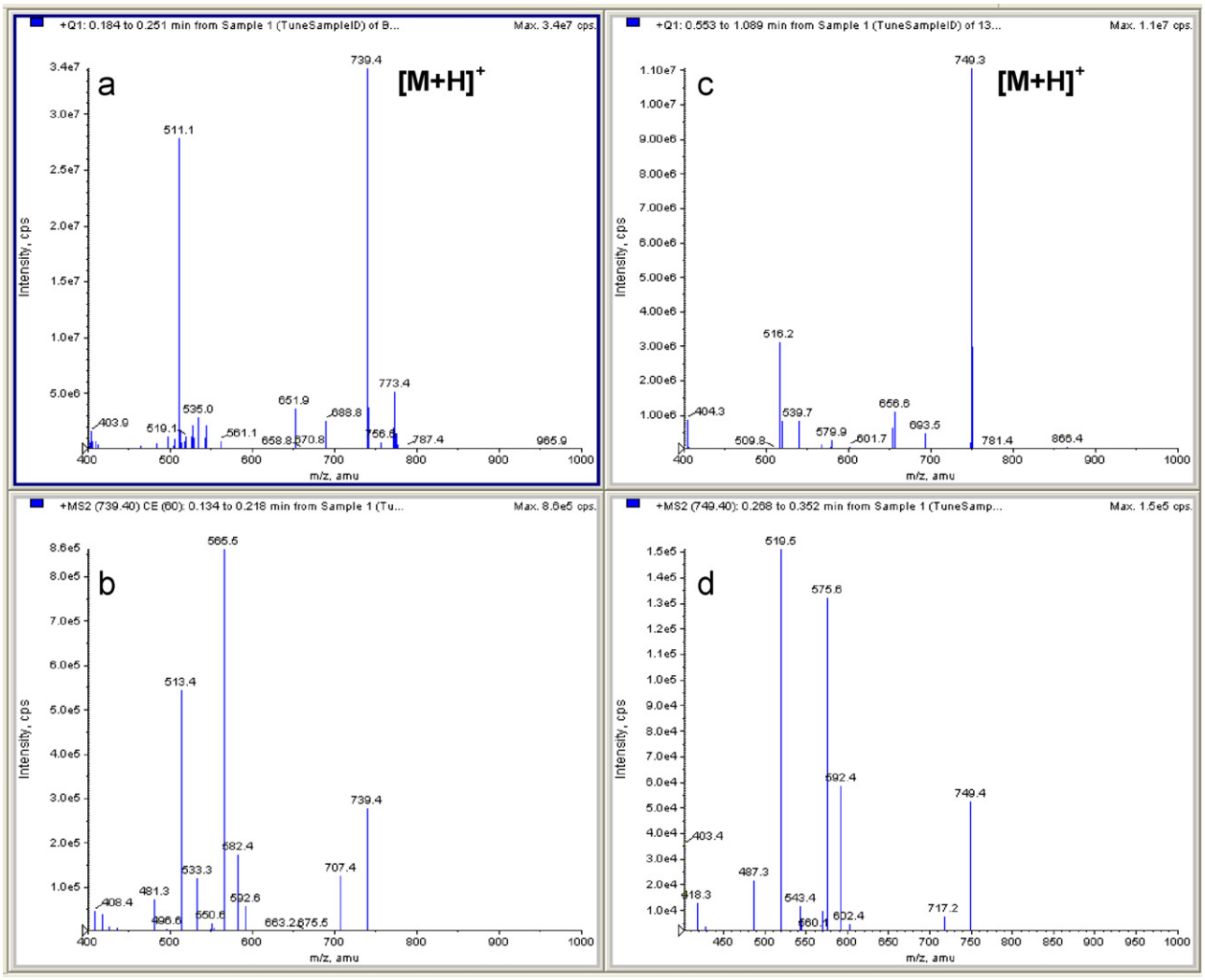

Fig. 2. Representative Q1 mass spectra and MS/MS product ion spectra of $[M+H]^+$ for BMS-790052 (m/z 739 > 565, a and b) and $^{13}C_{10}$-BMS-790052 (m/z 749 > 575, c and d).

screened for mobile phases at solution pHs 2.7, 4.0, 5.0, 6.5, and 10. The mobile phase screening was conducted on a XBridge C18 HPLC column (2 mm × 50 mm, 3 μm) using an isocratic program with 50% mobile phase A and 50% mobile phase B at a flow rate of 0.4 mL/min. Ammonium acetate (5 mM, pH 5.0) and acetonitrile provided the best chromatographic peak shape and response, and thus were selected as the mobile phases for column screening. In step 2, twenty reverse-phase HPLC columns were screened using a 3-min gradient program (20–60% mobile phase B) with the selected mobile phases from the Step 1. Atlantis dC18 column (2.1 mm × 50 mm, 5 μm) was selected based on an assessment that it provided the best peak shapes and responses. In step 3, confirmation of the suitability of the screened HPLC method (mobile phases and column) was performed by evaluating autosampler carryover, phospholipids profiling, metabolite interference in incurred samples, and internal standard consistency within a run. The autosampler carry-over issue was observed and successfully overcome by using the Shimadzu autosampler and a washing solution of isopropanol/methanol/water (40:40:20, v/v/v). Under this optimized HPLC condition, BMS-790052 was well separated from two metabolites, M2 and M4, and phospholipids in the incurred sample analysis, as shown in Fig. 4. The incurred samples were processed using acetonitrile precipitation method (sample: acetonitrile = 1:4, v/v). The metabolites, M2 and M4, were monitored in MRM mode at m/z 755 > 580, and m/z 681 > 339, respectively. Phospholipids profiles were monitored using negative precursor ion scan of m/z 153 and positive precursor ion scan of m/z 184) to detect different classes of phospholipids [11]. In step 4, sample extraction methods involving liquid–liquid extraction (LLE) and SPE were screened with the SPE method selected due to its lower matrix effect as compared to the LLE method, although recovery was not as good as the LLE method. In step 5, the screened extraction and LC–MS/MS method was confirmed by performing one accuracy and precision run which included six concentration levels of QCs in six replicates. Such systemic method screening strategy helps scientists to quickly find an optimal combination of HPLC mobile phases, columns, and extraction methods, which ensure the success of the method during validation and study sample analysis, and meet aggressive study timelines.

3.2. Linearity, accuracy and precision

Three accuracy and precision runs were performed in the full validation for the rat plasma assay. For the assays of dog, monkey, rabbit or mouse plasma, partial validations were conducted with one accuracy and precision run that included matrix-dependent stability tests to demonstrate the ruggedness of the assays. For all species, the standard curves were fitted to a $1/x^2$ weighted linear regression model with the range of 5.00–2000 ng/mL, Table 1 and Table 2. In each run, for at least three-fourths of the calibration stan-

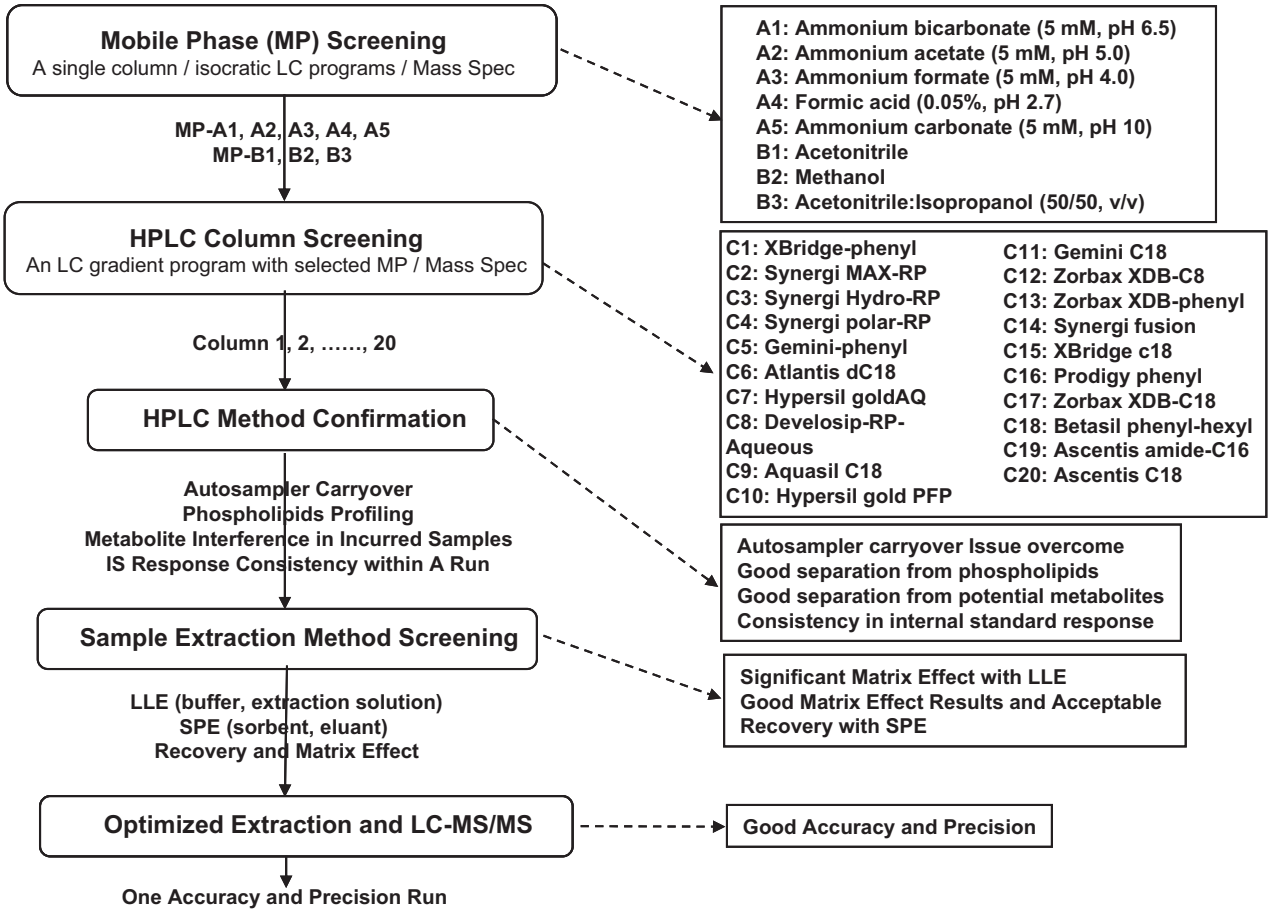

Fig. 3. Systematic method development workflow for quantitative determination of BMS-790052 in plasma.

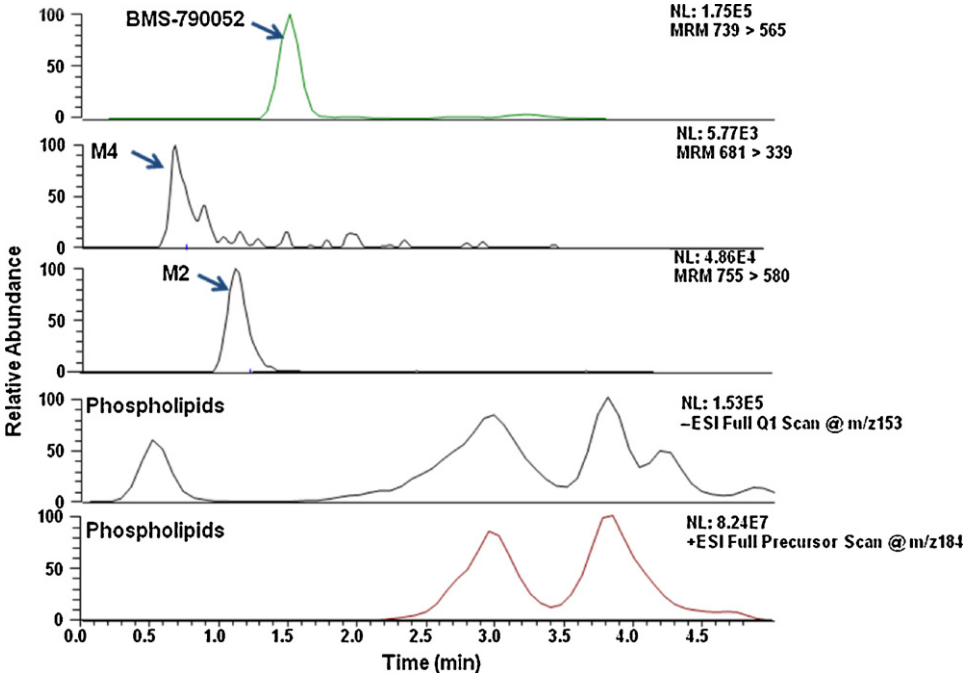

Fig. 4. Representative chromatograms of BMS-790052 metabolites and endogenous phospholipids in plasma.

Table 1
Accuracy (%Dev) of standard concentrations from nominal concentrations.

Conc. (ng/mL)	Rat		Dog		Monkey		Rabbit		Mouse	
	Mean %Dev $n=12$ run = 6	SD	Mean %Dev $n=6$ run = 3	SD	Mean %Dev $n=12$ run = 6	SD	Mean %Dev $n=10$ run = 5	SD	Mean %Dev $n=6$ run = 3	SD
5	−0.2	2.4	0.8	4.3	0.7	4.9	0.5	2.7	0.4	2.5
10	−0.8	2.3	0.6	9.2	−0.6	1.9	−1.1	2.4	−0.6	1.7
20	1.5	3.9	−3.0	2.8	−1.0	2.9	0.3	1.8	−0.1	4.9
50	3.4[a]	5.2	−1.9	2.9	−1.3	2.3	−0.5	2.4	−0.9	2.2
100	−0.8	3.2	−2.9	1.7	−1.0	2.5	0.0	2.0	−0.1	1.9
500	0.2	2.6	3.3	3.1	0.4	2.3	1.7	2.1	1.6	2.2
1000	−1.0	2.2	0.5	1.6	0.3	3.5	0.1	2.8	1.5	2.2
2000	−2.1	5.2	2.8	6.8	2.4	2.7	−1.2[b]	3.2	−1.8	2.0

[a] $n=11$, one standard was deactivated due to %Dev out of the acceptance criteria.
[b] $n=9$, one standard was deactivated due to %Dev out of the acceptance criteria.

Table 2
Standard curve regression analysis results ($r^2 > 0.99$).

	Rat		Dog		Monkey		Rabbit		Mouse	
	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept
Mean	0.010834	0.002185	0.010684	−0.002342	0.01353	−0.00254	0.009598	0.001413	0.009711	0.000734
SD	0.000909	0.003529	0.001162	0.008738	0.00416	0.00327	0.000153	0.001703	0.000091	0.000024
%CV	8.4	161.5	10.9	373.1	30.8	−128.5	1.6	120.5	0.9	3.3
n	6	6	3	3	6	6	4	4	2	2

Table 3
Accuracy and precision for BMS-790052 in plasma.

Species	QC Type (Nominal conc. in ng/mL)	LLOQ (5.00)	Low (15.0)	GM (125)	Mid (1000)	High (1600)	Dilution (100000)
Rat	Mean observed conc.	4.70	14.74	124.78	1008.00	1637.84	95285.74
	%Dev	−6.0	−1.7	−0.2	0.8	2.4	−4.7
	Between run precision (%CV)	0.0[a]	2.4	1.8	5.0	1.5	4.1
	Within run precision (%CV)	3.8	3.9	3.2	2.6	2.5	2.3
	Total variation (%CV)	3.8	4.6	3.7	5.6	2.9	4.7
	n	30	32	32	32	32	30
	Number of runs	5	6	6	6	6	5
Dog	Mean observed conc.	4.43	15.09	121.01	1013.99	1622.57	106678.83
	%Dev	−11.4	0.6	−3.2	1.4	1.4	6.7
	Between run precision (%CV)	N/A	7.9	6.9	8.5	5.2	N/A
	Within run precision (%CV)	4.5	4.9	3.1	2.4	3.4	3.8
	Total variation (%CV)	N/A	9.3	7.6	8.8	6.2	N/A
	n	6	9	10	10	10	6
	Number of runs	1	3	3	3	3	1
Monkey	Mean observed conc. in ng/mL	4.89	15.28	127.75	1018.32	1606.82	104352.85
	%Dev	−2.2	1.9	2.2	1.8	0.4	4.4
	Between run precision (%CV)	N/A	3.5	3.6	3.3	3.6	N/A
	Within run precision (%CV)	3.1	0.8	1.7	0.8	1.2	0.9
	Total variation (%CV)	N/A	3.6	4	3.4	3.8	N/A
	n	6	14	14	14	14	6
	Number of runs	1	5	5	5	5	1
Rabbit	Mean observed conc.	5.24	16.06	133.25	1054.39	1697.76	93713.91
	%Dev	4.8	7.1	6.6	5.4	6.1	−6.3
	Between run precision (%CV)	N/A	3.9	2.2	4.6	3.0	N/A
	Within run precision (%CV)	3.2	4.3	2.2	1.2	2.6	6.3
	Total variation (%CV)	N/A	5.7	3.1	4.8	4.0	N/A
	n	6	14	14	14	14	6
	Number of runs	1	5	5	5	5	1
Mouse	Mean observed conc.	4.61	14.39	121.58	984.49	1565.79	105370.15
	%Dev	−7.8	−4.1	−2.7	−1.6	−2.1	5.4
	Between run precision (%CV)	N/A	4.7	6.4	4.0	5.4	N/A
	Within run precision (%CV)	1.5	4.8	1.6	1.4	1.3	1.9
	Total variation (%CV)	N/A	6.7	6.6	4.3	5.5	N/A
	n	6	10	10	10	10	6
	Number of runs	1	3	3	3	3	1

NA, not applicable, because LLOQ QC and Dilution QC were only tested in one accuracy and precision run.
[a] No significant additional variation was observed as a result of performing the assay in different runs.

Table 4
Lower limit of quantification (LLOQ) for the determination of BMS-790052 in plasma.

Nominal conc. (ng/mL)	Rat		Dog		Monkey		Rabbit		Mouse	
	Pred. Conc.	% Dev	Pred. Conc.	% Dev	Pred. Conc.	% Dev	Pred. Conc.	% Dev	Pred. Conc.	% Dev
5	5.44	8.8	5.14	2.8	5.32	6.4	4.49	−10.2	4.86	−2.8
	5.27	5.4	4.86	−2.8	4.69	−6.2	4.76	−4.8	4.60	−8.0
	5.27	5.4	4.86	−2.8	4.72	−5.6	4.43	−11.4	4.81	−3.8
	5.21	4.2	4.84	−3.2	4.57	−8.6	4.62	−7.6	4.89	−2.2
	5.03	0.6	4.53	−9.4	4.75	−5.0	4.15	−17.0	4.94	−1.2
	4.84	−3.2	4.66	−6.8	4.67	−6.6	4.70	−6.0	4.85	−3.0
Mean Conc.	5.18		4.82		4.79		4.53		4.83	
SD	0.21		0.21		0.27		0.22		0.12	
%CV	4.1		4.3		5.6		4.9		2.5	

dards, the deviations of the back-calculated concentrations from their nominal values were within ±15.0% (±20.0% at the LLOQ level). Correlation coefficient values (r^2) of the standard curves were all greater than 0.99. The accuracy and precision information were obtained using a one-way analysis of variance (ANOVA) in the Watson LIMS (version 7.4, Thermo Scientific Inc.). The intra-assay precision (%CV), based on four levels of analytical QCs (Low, GM, Mid and High), was within 4.9%; inter-assay precision (%CV) was within 8.5% for all species, and the mean assay accuracy (%Dev) was within ±7.1% for all species, Table 3.

3.3. Lower limit of quantitation and selectivity

The LLOQ for the analyte was assessed using plasma samples at 5.00 ng/mL for BMS-790052. Six different lots of drug-free K_2EDTA plasma from each species were spiked to obtain the six LLOQ samples. The deviations of the six predicted BMS-790052 concentrations from the nominal values were within ±8.8% in rat plasma, ±9.4% in dog plasma, ±8.6% in monkey plasma, ±17.0 in rabbit plasma, and ±8.0 in mouse plasma, Table 4. Representative MRM chromatograms at the LLOQ concentration are shown in Fig. 5.

Six different lots of drug-free K_2EDTA plasma from each species were analyzed with and without the internal standard in order to determine whether any endogenous plasma constituents interfered with the analyte or the internal standard. The degree of interference was assessed by inspection of MRM chromatograms. As shown in Fig. 5, no significant interfering peaks from the plasma were found at the retention time and in the ion channels of either the analyte or the internal standard. In addition, for QC0 samples (blank matrix sample processed with internal standard) and LLOQ samples, the response ratio was obtained by dividing the response (peak area) of the analyte by the response (peak area) of the internal standard. For each lot of matrix, a subsequent ratio was calculated by dividing the response ratio of the LLOQ sample by the response ratio of the corresponding QC0 sample. The ratio values obtained for each LLOQ sample were all greater than 16.4 where the ratio of LLOQ/QC0 ≥ 5 was the acceptance criteria, which demonstrated the good selectivity at the LLOQ, Table 5.

3.4. Recovery and matrix effect

The extraction recoveries of BMS-790052 in the plasma from different species were determined at 15.0 and 1600 ng/mL for

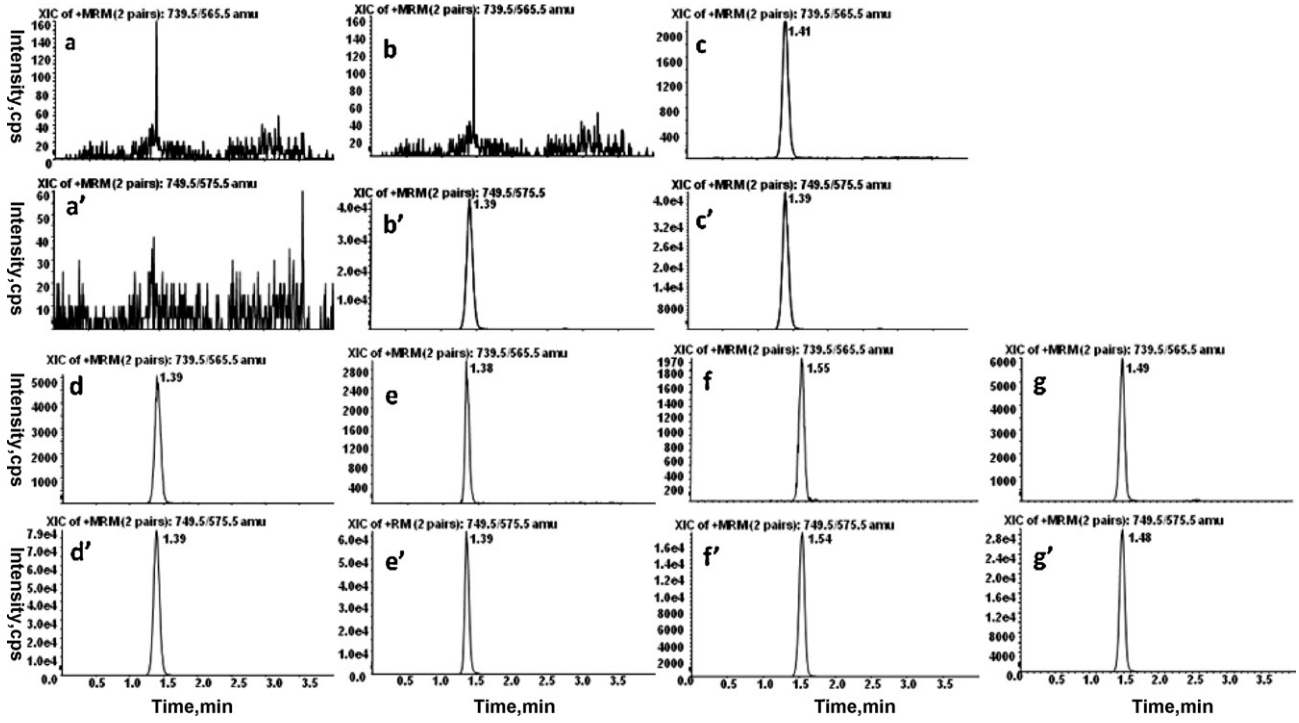

Fig. 5. Representative chromatograms of BMS-790052 and $^{13}C_{10}$-BMS-790052 (IS) in blank-blank (a and a′), blank-IS (b and b′), LLOQ (c and c′) in rat plasma, and LLOQ in dog plasma (d and d′), monkey plasma (e and e′), rabbit plasma (f and f′), and mouse plasma (g and g′).

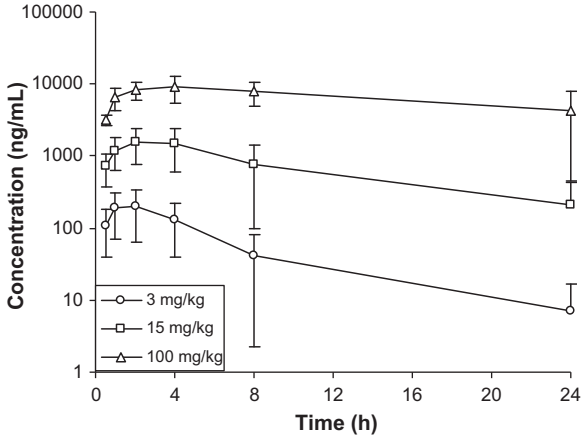

Fig. 6. Representative toxicokinetic profiles of BMS-790052 in a dog study.

BMS-790052 by comparing the response ratios in the plasma samples spiked with BMS-790052 prior to extraction with those spiked post-extraction. The extraction recoveries of the internal standard were determined similarly at 100 ng/mL. The experiment was conducted in three replicated. The recoveries of BMS-790052 in the plasma from different species were low at ~30% but consistent at both concentrations of 15.0 and 1600 ng/mL (data not shown), Table 6. Low recoveries were obtained because strong SPE washing solution (50% acetonitrile in water) was necessary to wash off endogenous interference before eluting.

The matrix effect was expressed as matrix factor (MF), i.e., MF = 1 indicates no matrix effects, MF < 1 indicates ion suppression, and MF > 1 indicates ion enhancement. The MFs were determined at concentrations of 15.0 and 1600 ng/mL for BMS-790052 in the plasma from different species by dividing the analyte response (peak area) in the plasma sample spiked post-extraction with BMS-790052 by the analyte response (peak area) of those spiked in reconstitution solution. The matrix effect of the internal standard was determined similarly at 100 ng/mL. The matrix factors for plasma in different species were within 0.86–1.08, indicating no significant matrix effect on BMS-790052 detection, Table 6.

3.5. Stability

The room temperature, freeze-thaw, and frozen storage stabilities of BMS-790052 in plasma from different species were evaluated in triplicate by using QCs (Low, High, and Dilution QC). Deviations of mean predicted concentrations of test samples from nominal concentrations were calculated as an indicator of the stability of the analyte. Based on the results, BMS-790052 was stable for at least 24 h at room temperature and at least five freeze-thaw cycles in rat, dog, monkey, rabbit, and mouse plasma. Stability in plasma was demonstrated at ~−20 °C for at

Table 5
Evaluation of LLOQ response to background response (LLOQ/QC0 ratio) for BMS-790052 in six different lots plasma.

Rat	Dog	Monkey	Rabbit	Mouse
77.1	16.6	145.6	110.3	137.9
36.4	42.0	413.9	> 5.0[a]	83.1
68.4	17.7	61.7	63.6	42.6
38.5	16.4	114.0	> 5.0[a]	72.7
60.4	34.1	115.6	84.4	52.7
>5.0[a]	36.8	124.1	461.0	82.2

[a] QC0 response was zero.

Table 6
Recovery and matrix effect.

Analyte	Rat		Dog		Monkey		Rabbit		Mouse	
	Recovery %	Matrix factor (MF)	Recovery %	Matrix factor (MF)	Recovery %	Matrix factor (MF)	Recovery %	Matrix factor (MF)	Recovery %	Matrix factor (MF)
BMS-790052	29.4–38.2	0.90–1.00	28.3–28.6	1.01–1.06	29.6–32.1	1.03–1.04	26.3–26.4	1.01–1.06	34.5–37.1	1.05–1.08
$^{13}C_{10}$-BMS-790052	28.1–56.6	0.86–0.99	26.2–61.6	0.99–1.04	46.4–46.5	1.03–1.05	34.2–36.1	0.96–1.02	37.6–39.9	1.00–1.06

Table 7
Result of incurred sample reproducibility testing in a rat toxicokinetic study.

Sample ID[a]	Initial value (ng/mL)	Incurred repeat (ng/mL)	Mean (ng/mL)	%Dev from mean[b]
Rat 1, 2 M, 40 mg/kg, Day 1 1 h	20190.30	19879.61	20034.96	0.8
Rat 2, 2 M, 40 mg/kg, Day 1 4 h	10444.21	10498.57	10471.39	0.3
Rat 3, 2 M, 40 mg/kg, Day 14 1 h	18691.17	18357.56	18524.37	0.9
Rat 4, 2 M, 40 mg/kg, Day 14 4 h	7840.40	7837.40	7838.90	0.0
Rat 13, 2 F, 40 mg/kg, Day 1 1 h	20750.95	20025.44	20388.20	1.8
Rat 14, 2 F, 40 mg/kg, Day 14 2 h	11852.50	12236.36	12044.43	1.6
Rat 15, 2 F, 40 mg/kg, Day 14 8 h	3285.70	3374.89	3330.30	1.3
Rat 5, 3 M, 400 mg/kg, Day 1 2 h	66441.24	71484.93	68963.09	3.7
Rat 6, 3 M, 400 mg/kg, Day 1 24 h	5685.99	5657.42	5671.71	0.3
Rat 7, 3 M, 400 mg/kg, Day 14 1 h	64155.11	64959.25	64557.18	0.6
Rat 9, 3 M, 400 mg/kg, Day 14 4 h	89042.81	86992.65	88017.73	1.2
Rat 13, 3 F, 400 mg/kg, Day 1 1 h	67559.76	70128.06	68843.91	1.9
Rat 16, 3 F, 400 mg/kg, Day 1 2 h	50890.37	53808.65	52349.51	2.8
Rat 17, 3 F, 400 mg/kg, Day 14 4 h	82484.53	88242.43	85363.48	3.4
Rat 8, 4 M, 1500 mg/kg, Day 1 4 h	151559.29	145339.11	148449.20	2.1
Rat 10, 4 M, 1500 mg/kg, Day 1 8 h	187852.06	188204.52	188028.29	0.1
Rat 11, 4 M, 1500 mg/kg, Day 14 8 h	147592.84	149619.46	148606.15	0.7
Rat 12, 4 M, 1500 mg/kg, Day 14 24 h	80144.98	80949.38	80547.18	0.5
Rat 18, 4 F, 1500 mg/kg, Day 1 4 h	180862.26	185092.03	182977.15	1.2
Rat 19, 4 F, 1500 mg/kg, Day 1 24 h	24614.58	23953.35	24283.97	1.4
Rat 14, 4 F, 1500 mg/kg, Day 14 2 h	79314.40	78783.99	79049.20	0.3
Rat 20, 4 F, 1500 mg/kg, Day 14 8 h	164963.88	165329.30	165146.59	0.1

[a] Sample ID listed as: animal number, treatment (group number and sex; M, male; F, female), dose, and collection time.
[b] 10% as the acceptance criteria.

least 287 days in rat plasma, 74 days in dog plasma, 137 days in monkey plasma, 92 days in rabbit plasma, and 92 days in mouse plasma. The processed plasma samples were stable for 56 h in the autosampler at ~5 °C. The stabilities of the freshly prepared stock solutions of BMS-790052 and the internal standard, stored at room temperature and at ~4 °C, were evaluated in replicates of six after appropriate dilutions. The results showed that the analyte and internal standard were stable in methanol for at least 6 h at room temperature, and at least 84 days at ~4 °C.

3.6. Carryover

The carryover of BMS-790052 in the rat plasma assay was tested by analyzing in triplicate a blank sample right after each High QC (1600 ng/mL). The carryover was calculated as the percent response in the blank compared to the response in the High QC sample and was observed to be 0.0091%, which represents ~0.2 ng/mL at the assay ULOQ and would have no impact on samples at or near the LLOQ. The carryover of the internal standard was determined at 100 ng/mL in a similar way, and no carryover was observed.

3.7. Batch size

It has been demonstrated that the method performs well for a batch size of up to 192 samples, including calibration standards and QCs, without observing abnormal chromatography or spectrometry.

3.8. Determination of BMS-790052 concentrations in plasma

This method had been used to analyze several thousand plasma samples from toxicokinetic studies in rats, dogs, monkeys, rabbits, and mice. Pharmacokinetic parameters were derived from plasma concentration-time data by a non-compartmental method using Enterprise Pharmacology (EP) Series™ and Kinetica™ (Thermo Electron Corp.). The comparable analytical data from different toxicokinetic studies confirmed the method's ruggedness, selectivity, and reproducibility, which are essential for delivering high quality bioanalytical data. A representative toxicokinetic profile in a dog study was shown in Fig. 6.

3.9. Incurred sample reanalysis

The incurred sample reproducibility (ISR) for plasma samples from each species (rat, dog, monkey, rabbit, and mouse) was evaluated by re-analyzing incurred study samples with a minimum of twenty or > 5% of total study samples. The representative incurred samples were selected from different dose groups, sex, and sample collection times in each study. Assay reproducibility for each species was demonstrated based on the results that each of a sample's results (initial or repeat) was within 10.0% of the mean of the two values for two-thirds (2/3) of the samples tested. The representative testing results for the mouse plasma assay were shown in Table 7. The %Deviation from each mean value for all of the twenty-two samples was within 3.7%, demonstrating good assay reproducibility.

4. Conclusions

A rugged, accurate and sensitive LC–MS/MS method for the quantitation of BMS-790052 in 50 μL of plasma from rats, dogs, monkeys, rabbits, and mice has been developed and validated over the concentration range of 5.00–2000 ng/mL.

References

[1] M. Gao, R.E. Nettles, M. Belema, L.B. Snyder, V.N. Nguyen, R.A. Fridell, M.H. Serrano-Wu, D.R. Langley, J.H. Sun, D.R. O'Boyle 2nd, J.A. Lemm, C. Wang, J.O. Knipe, C. Chien, R.J. Colonno, D.M. Grasela, N.A. Meanwell, L.G. Hamann, Nature 465 (2010) 96.
[2] M.W. Fried, M.L. Shiffman, K.R. Reddy, C. Smith, G. Marinos, F.L. Gonçales Jr., D. Häussinger, M. Diago, G. Carosi, D. Dhumeaux, A. Craxi, A. Lin, J. Hoffman, J. Yu, N. Engl. J. Med. 347 (2002) 975.
[3] S.J. Hadziyannis, H. Sette Jr., T.R. Morgan, V. Balan, M. Diago, P. Marcellin, G. Ramadori, H. Bodenheimer Jr., D. Bernstein, M. Rizzetto, S. Zeuzem, P.J. Pockros, A. Lin, A.M. Ackrill, Ann. Intern. Med. 140 (2004) 346.
[4] A.J. Muir, J.D. Bornstein, P.G. Killenberg, N. Engl. J. Med. 350 (2004) 2265.
[5] M. Jemal, Y.Q. Xia., Curr. Drug Metab. 7 (2006) 491.
[6] M.S. Lee, E.H. Kems, Mass Spectrom. Rev. 18 (1999) 187.

[7] J. Ayrton, G.J. Dear, W.J. Leavens, D.N. Mallett, R.S. Plumb, J. Chromatogr. B Biomed. Appl. 709 (1998) 243.
[8] J. Ayrton, R. Plumb, W.J. Leavens, D. Mallett, M. Dickins, G.J. Dear, Rapid Commun. Mass Spectrom. 12 (1998) 217.
[9] M. Jemal, Z. Ouyang, Y. Xia, Biomed. Chromatogr. 24 (2010) 2.
[10] Guidance for Industry: Bioanalytical Method Validation, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf.
[11] Y. Xia, M. Jemal, Rapid Commun. Mass Spectrom. 23 (2009) 2125.