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Validation and implementation of a liquid chromatography/tandem mass spectrometry assay to quantitate ABT-751, ABT-751 glucuronide, and ABT-751 sulfate in human plasma for clinical pharmacology studies

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Abstract

A method has been developed for the quantitation of ABT-751, ABT-751 glucuronide, and ABT-751 sulfate in human plasma. ABT-751 and metabolites were separated from endogenous material on a C18 column with acetonitrile–ammonium acetate (2 mM) mobile phase containing formic acid (0.1%, v/v) using isocratic flow for 5 min. The analytes were monitored by tandem-mass spectrometry. Calibration curves were generated over the range of 20–5000 ng/ml for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate. A 20,000 ng/ml sample that was diluted 1:10 (v/v) with plasma was accurately quantitated. The method has been successfully applied to study the plasma pharmacokinetics of ABT-751 in humans.

Keywords: ABT-751; LC/MS/MS; Pharmacokinetics

1. Introduction

ABT-751 is an orally bioavailable sulfonamide with antimitotic properties by binding to the colchicines binding site on β-tubulin [1,2]. Pre-clinical activity has been observed in various solid tumors rodent models and human tumor xenografts [3]. Activity has been noted in P-glycoprotein overexpressed multidrug resistance cell lines [3]. The mechanism of action, oral formulation, and favorable anti-tumor activity in pre-clinical models lead to the clinical development of ABT-751 as an anticancer agent.

Early dose-ranging studies determined that ABT-751 was safe and achieved concentrations associated with pre-clinical efficacy [2]. ABT-751 was rapidly absorbed with the maximum concentration (C_{max}) achieved within 3 h of administration and had a half-life ranging from 4.4 to 16.6 h [4]. Two inactive ABT-751 metabolites, ABT-751 glucuronide and ABT-751 sulfate, were identified in vivo from patient receiving oral ABT-751 [2,4].

ABT-751 is currently being evaluated in Phase I clinical trials in patients with advanced colorectal cancers with the drug being administered once daily alone or in combination with XELIRI (capecitabine and irinotecan) plus bevacizumab at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. A HPLC/UV method quantitated ABT-751 from 50 to 5000 ng/ml in plasma but did not describe the calibration range utilized for ABT-751 glucuronide or ABT-751 sulfate [4]. This HPLC/UV method utilized 250 μl of plasma with a complex liquid–liquid extraction and a run time of greater than 10 min. Based on preliminary reports from other clinical trials, the calibration range should be extended to include 20,000 ng/ml for the dosing schedule being employed in the colorectal trial at SKCCC at Johns Hopkins [4–6]. In order to comprehensively characterize the clinical pharmacology of ABT-751, a method for the quantitation of ABT-751 and its metabolites in plasma was necessary. ABT-751, ABT-751 glucuronide, and

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ABT-751 sulfate were quantitated in a clinically relevant range in plasma by LC/MS/MS to achieve a rapid and specific assay method.

2. Experimental

2.1. Chemical and reagents

ABT-751, ABT-751 glucuronide, ABT-751 sulfate, and the internal standard, A-93387, were a gift from Abbott Laboratories (Abbott Park, IL, USA). Ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ, USA). Formic acid (98%, v/v in water) and HPLC grade acetonitrile were obtained from EMD Chemicals Inc. (Gibbstown, NJ, USA). Ortho-phosphoric acid (85%, v/v) was purchased from Fluka (Ronkondoma, NY, USA). Deionized water was obtained from a Milli-Q-UF system (Millipore, Milford, MA, USA) and used in all aqueous solutions. Drug-free (blank) human plasma containing EDTA was purchased from Biological Specialty Corporation (Colmar, PA, USA).

2.2. Preparation of stock solutions

Stock solutions of ABT-751, ABT-751 glucuronide, and ABT-751 sulfate were prepared in duplicate as 1 mg/ml solutions in acetonitrile–water (1:1, v/v). The area counts for each of the duplicated aliquots were 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. ABT-751 glucuronide was unstable when repeatedly removed from the freezer and was subsequently stored in single-use aliquots. Stock solutions of ABT-751, ABT-751 glucuronide (single-use aliquots), and ABT-751 sulfate were stable for 3 months.

A stock solution of A-93387 was prepared by dissolving 2 mg, accurately weighed, in 2 ml of acetonitrile–water (1:1, v/v). The 1 mg/ml solution was stored in a glass vial at −20 °C.

2.2.1. Preparation of calibration standards and quality controls in plasma

ABT-751, ABT-751 glucuronide, and ABT-751 sulfate 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. Eight standards were prepared including the concentrations 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/ml, and four QC were prepared at concentrations 20 (lower limit of quantitation (LLOQ)), 60, 300, and 4000 ng/ml. Since the calibration curve did not achieve the range intended, an additional dilutional QC was prepared at 20,000 ng/ml and diluted 1:10 (v/v) in pooled human plasma for quantitation. All standards and QC samples were prepared fresh daily. For long-term and freeze-thaw stability, QC samples were prepared as a batch and stored at −70 °C.

A 10 μl aliquot of the 1 mg/ml internal standard stock solution was added into 250 ml of acetonitrile for a final concentration of 40 ng/ml at the time of analysis.

2.3. Sample preparation

Prior to extraction, frozen plasma samples were thawed in a water bath at ambient temperature. A 100 μl aliquot of plasma was added to a borosilicate glass tube (13 mm × 100 mm) followed by 100 μl of ortho-phosphoric acid–water (5:95, v/v). The tube was mixed vigorously for 10 s on a vortex-mixer after which 300 μl of acetonitrile containing 40 ng/ml A-93387 was added. 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 supernatant (100 μl) was transferred to a borosilicate glass tube (13 mm × 100 mm) to which 100 μl of water was added. After being mixed vigorously for 30 s on a vortex-mixer, the contents were transferred to a 250 μl polypropylene autosampler vial, and a volume of 10 μl was injected onto the LC/MS/MS instrument for quantitative analysis using an autosampling device.

2.4. Equipment

Chromatographic analysis was performed using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA). The HPLC system consisted of a binary pump, a vacuum degassing unit, and a refrigerated autosampler operating at 10 °C. Separation of the analyte from potentially interfering material was achieved at ambient temperature using Waters X-Terra MS C18 column (50 mm × 2.1 mm, i.d.) packed with a 3.5 μm stationary phase, protected by a guard column packed with 3.5 μm RP18 material (Milford, MA, USA). The mobile phase used for the chromatographic separation was composed of acetonitrile–ammonium acetate (pH 2.9; 2 mM) (60:40, v/v) containing formic acid (0.1%, v/v), and was delivered isocratically at a flow rate of 0.15 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 positive mode and controlled by the Analyst version 1.2 software (Applied Biosystems). Samples were introduced into the interface through a Turbo IonSpray temperature set at 400 °C. A high positive voltage of 5.0 kV was applied to the ion spray. Nitrogen was used as the nebulizer gas, curtain gas, and collision gas with the settings of 12, 8, and 10, respectively. Other optimal parameters included declustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) are reported in Table 1. The spectrometer was programmed to allow the [M + H] ion of ABT-751, ABT-751 glucuronide, and ABT-751 sulfate, and the internal standard at *m/z* 372.1, 548.2, 452.0, and 500.3,

Table 1
Optimization parameters for ABT-751 and metabolites

	DP (V)	FP (V)	EP (V)	CE (eV)	CXP (V)
ABT-751	46	250	10	47	14
ABT-751 glucuronide	41	210	10	75	14
ABT-751 sulfate	51	310	10	53	14
A-93387	41	230	10	37	8

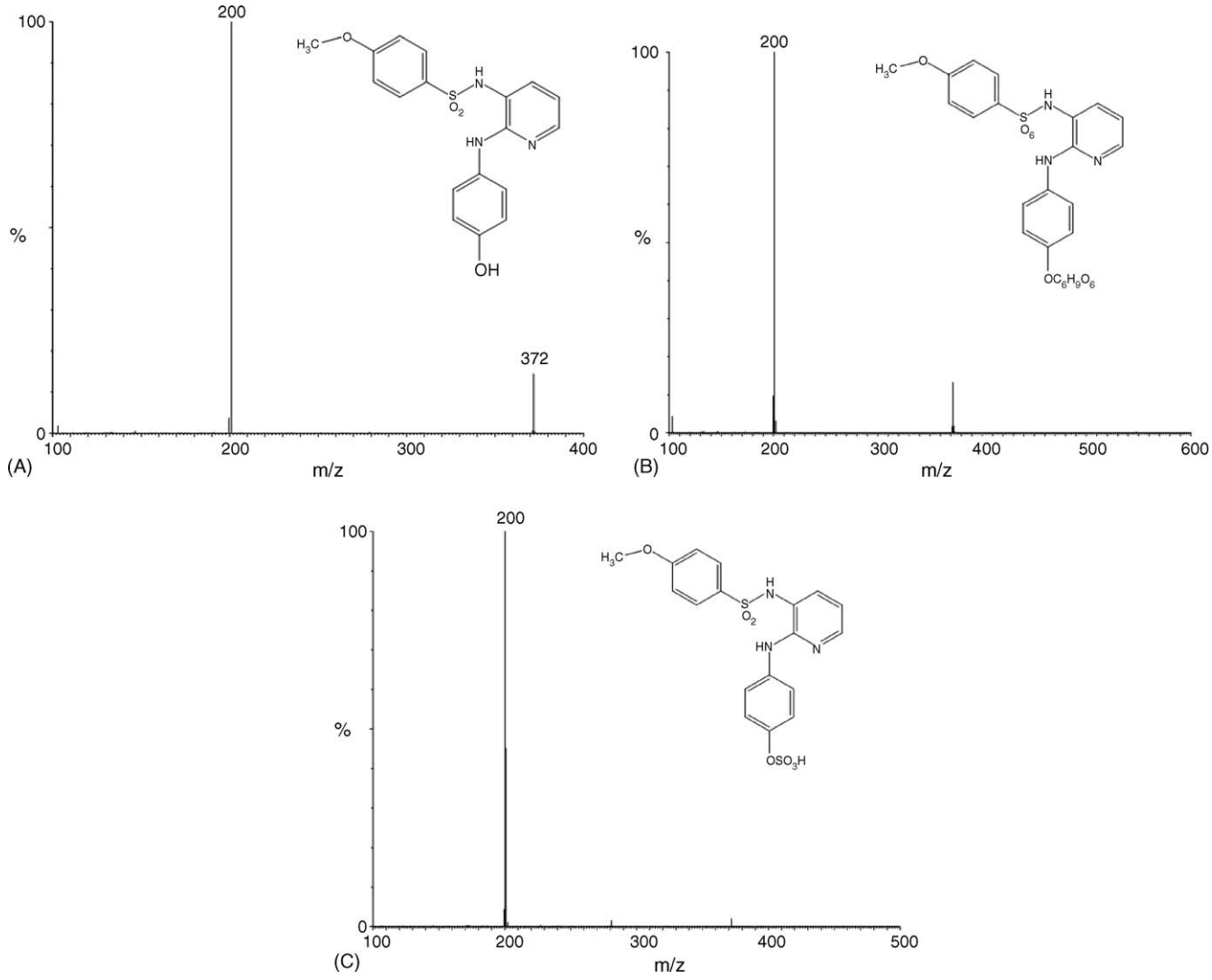

Fig. 1. Full-scan product ion spectrum and chemical structure for ABT-751 (A), ABT-751 glucuronide (B), and ABT-751 sulfate (C).

respectively, to pass through the first quadrupole (Q1) and into the collision cell (Q2). The product ions ABT-751, ABT-751 glucuronide, ABT-751 sulfate, and the internal standard at *m/z* 200.0, 199.8, 199.8, and 272.0, respectively, were monitored through the third quadrupole (Q3) (Fig. 1). The mass spectrometer has a resolution of 0.2 amu. The dwell time per channel was 250 ms for data collection.

2.5. Calibration curves

2.5.1. Plasma

ABT-751, ABT-751 glucuronide, and ABT-751 sulfate calibration samples were prepared in plasma over the range of 20–5000 ng/ml. Calibration curves were computed by the Analyst software using the ratio of the peak area of the analyte and internal standard by quadratic regression using 1/[nominal concentration] weight. 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. Validation procedures

2.6.1. Pre-study validation

Method validation runs were performed on 4 days. Each analytical run consisted of a calibration curve using duplicate standards at each concentration and QC samples in quintuplicate. The accuracy and precision of the assay was assessed by the mean relative percentage deviation (DEV) from the nominal concentrations and the within-run and between-run precision, respectively. The accuracy for each tested concentration was calculated as:

$$\mathrm{DEV}_{(\mathrm{analyte})}\,(\%) = \left\{\left([\mathrm{analyte}]_{\mathrm{mean}} - [\mathrm{analyte}]_{\mathrm{nominal}}\right) / [\mathrm{analyte}]_{\mathrm{nominal}}\right\} \times 100$$

Estimates of the between-run precision were obtained by one-way analysis of variance (ANOVA) using the run day as the classification variable. The between-groups mean square (MS_{bet}), the within-groups mean square (MS_{wit}), and the grand mean (GM) of the observed concentrations across runs were calcu-

lated using the JMP™ statistical discovery software version 4 (SAS Institute, Cary, NC, USA). The between-run precision (BRP), expressed as a percentage relative standard deviation, was defined as:

$$\mathrm{BRP}\,(\%) = \left(\sqrt{((\mathrm{MS_{bet}} - \mathrm{MS_{wit}})/n)}/\mathrm{GM}\right) \times 100$$

where n represents the number of replicate observations within each run. For each concentration, the estimate of the within-run precision (WRP) was calculated as:

$$\mathrm{WRP}\,(\%) = \left(\sqrt{(\mathrm{MS_{wit}})}/\mathrm{GM}\right) \times 100$$

The specificity of the method was tested by visual inspection of chromatograms of extracted human plasma samples from six different donors for the presence of endogenous or exogenous interfering peaks. The peak area needed to be less than 20% than the peak area for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate at 20 ng/ml in an aqueous solution. If not, plasma from six additional donors would be tested. In addition, analytical specificity was determined by injecting either a neat solution or plasma sample spiked with either ABT-751 glucuronide at 4000 ng/ml or ABT-751 sulfate at 4000 ng/ml and monitoring the signal on all transitions. The relative recovery of the assay was measured by comparison of the mean concentration values of extracted plasma samples in triplicate and aqueous samples at 60 and 4000 ng/ml. Autosampler stability was also assessed at 60 and 4000 ng/ml with continuous injection of samples for approximately 10 h. The short-term stability plasma was assessed at 60 and 4000 ng/ml in triplicate on the benchtop for 1, 2, 4 and 6 h. The stability of ABT-751 and metabolites in plasma was tested at 60, 300, and 4000 ng/ml in triplicate by three freeze-thaw cycles at −70 °C. The long-term stability test was assessed at 60, 300, and 4000 ng/ml in triplicate at −70 °C at 11 and 109 days. The mean values of the triplicate samples were compared

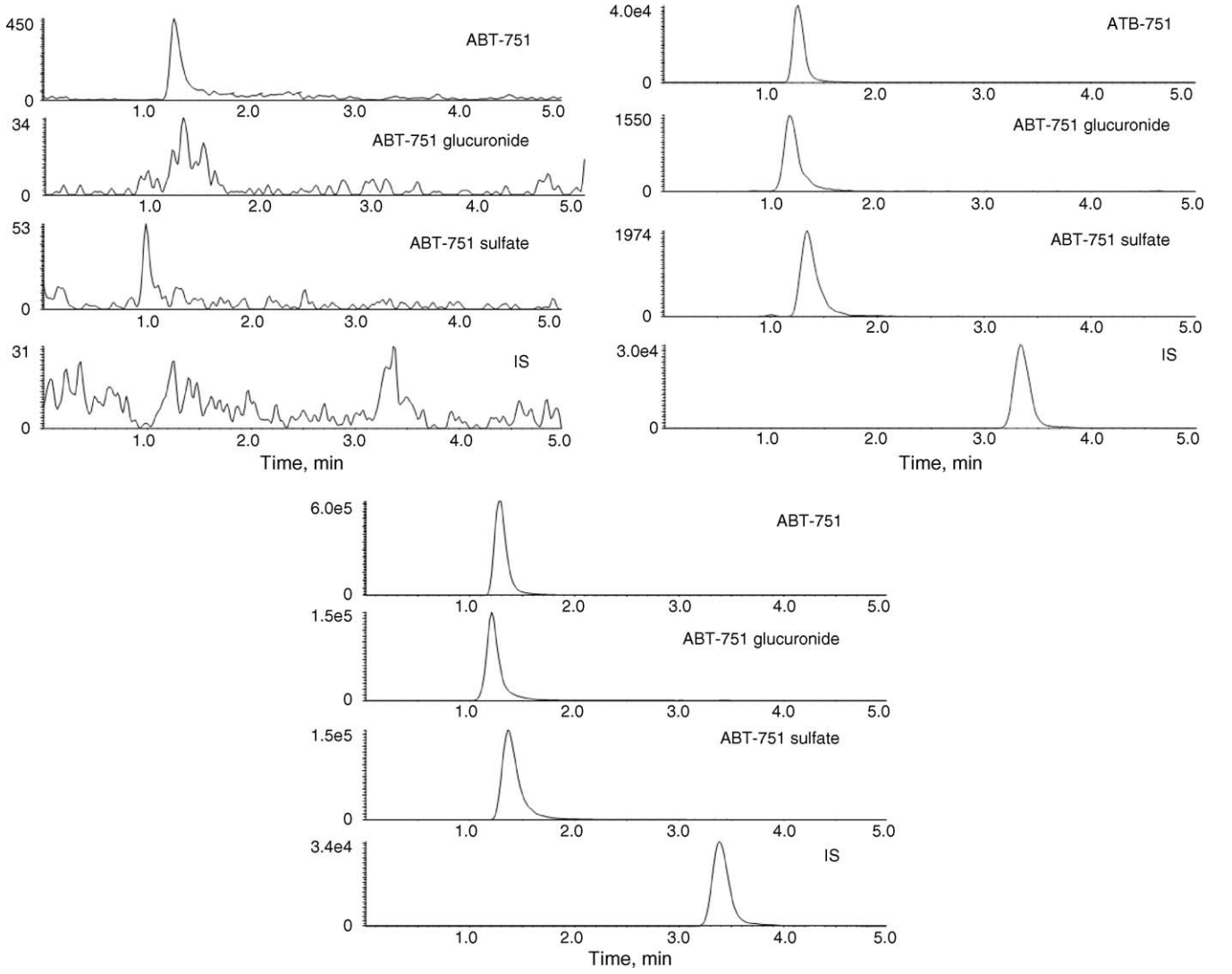

Fig. 2. Selected ion chromatograms of blank plasma (A), plasma spiked with concentrations at the LLOQ for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate with retention times of 1.30, 1.20, and 1.30 min, respectively (B), and a select patient sample obtained 8 h after the oral administration of 150 mg of ABT-751 (C). The following mass-to-charge (m/z) transitions were monitored 372.1 → 200.0 for ABT-751, 548.2 → 199.8 for ABT-751 glucuronide, 452.0 → 199.8 for ABT-751 sulfate, and 500.3 → 272.0 for internal standard.

to the initial nominal concentrations for short-term, long-term, and freeze-thaw stability.

2.6.2. Cross-validation

Abbott Laboratories (Abbott Park, Illinois) provided blinded plasma samples containing EDTA that were spiked with varying amounts of ABT-751, ABT-751 glucuronide, and ABT-751 sulfate to mimic a patient who would be treated with ABT-751. Samples were analyzed according to the analytical methods described within this manuscript and by Abbott. The Abbott methodology involved isolation of the analytes from plasma by solid phase extraction with analysis by reversed phase HPLC and MS/MS detection [7].

2.6.3. In-study validation

Samples from patients receiving treatment with ABT-751 were analyzed over a time period involving 5 separate days. Each analytical run consisted of a calibration curve using single standards at each concentration except duplicate standards were analyzed for the LLOQ, upper limit of quantitation (ULQ), and QC samples in duplicate or triplicate if more than 40 unknown samples were analyzed. The accuracy and precision of the assay were assessed by the same methodology as the plasma samples (see Section 2.6.1).

2.7. Pharmacokinetic analysis

ABT-751 is currently being evaluated in a Phase I clinical trial in patients with advanced colorectal cancers. The patient studied participated in this study and received a dose of ABT-751 of 150 mg administrated orally once daily for 7 days alone and for 14 days in combination with XELIRI (capecitabine and irinotecan) plus bevacizumab. Pharmacokinetics were assessed when ABT-751 was administered alone or in combination at steady-state. The drug was formulated as 25 and 100 mg capsules containing povidone, microcrystalline cellulose, lactose

Table 2
Back-calculated concentrations from calibration curves for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate in human plasma

Nominal concentration (ng/ml)	*n*	Accuracy (%)	Concentration (ng/ml)[a]	Precision (%)	
				Within-run	Between-run
ABT-751					
20	8	97.1	19.4 ± 1.8	4.0	9.2
50	8	96.3	48.1 ± 3.1	3.1	6.1
100	8	104.6	104.6 ± 4.3	4.6	–[b]
200	8	102.4	204.9 ± 7.5	3.6	0.5
500	8	99.6	498.0 ± 24.4	3.4	3.8
1000	8	101.9	1018.9 ± 36.8	3.3	1.6
2000	8	98.3	1965.0 ± 83.7	2.7	3.6
5000	8	100.6	5031.3 ± 240.4	6.2	–[b]
ABT-751 glucuronide					
20	8	95.6	19.1 ± 1.3	1.2	7.0
50	8	97.5	48.8 ± 1.5	1.3	2.9
100	8	107.4	107.4 ± 3.5	3.0	1.4
200	8	99.6	199.1 ± 10.5	2.9	4.8
500	8	97.8	488.8 ± 23.7	4.9	–[b]
1000	8	105.2	1051.5 ± 38.9	2.6	2.8
2000	8	96.8	1936.3 ± 90.7	4.5	1.4
5000	8	100.5	5025.0 ± 169.0	4.4	–[b]
ABT-751 sulfate					
20	8	92.6	18.5 ± 1.6	4.8	7.7
50	8	98.0	49.0 ± 2.8	4.5	3.8
100	8	108.0	108.0 ± 4.1	3.6	1.4
200	8	102.3	204.5 ± 10.7	3.3	4.4
500	8	98.0	490.1 ± 20.1	5.4	–[b]
1000	8	103.6	1035.6 ± 47.9	3.4	3.4
2000	8	97.4	1948.8 ± 108.4	5.9	–[b]
5000	8	100.7	5033.8 ± 223.7	5.8	–[b]

[a] Values are mean ± standard deviation.
[b] No significant additional variation was observed as a result of performing the assay in different runs.

Table 3
Assessment of accuracy, precision, and recovery in plasma

Nominal concentration (ng/ml)	*n*	Accuracy (%)	Precision (%)		Recovery (%)
			Within-run	Between-run	
ABT-751 validation					
20	20	93.6	6.0	7.7	ND[a]
60	20	99.5	4.3	8.0	100.8
300	20	100.6	4.3	4.4	ND[a]
4000	19	98.0	3.3	2.7	90.3
20000	20	101.7	3.0	8.3	ND[a]
(1:10)[b]					
ABT-751 glucuronide validation					
20	20	99.2	5.7	8.3	ND[a]
60	20	98.8	5.3	5.4	30.6
300	20	94.1	4.2	1.1	ND[a]
4000	19	97.6	3.4	2.8	29.6
20000	20	99.9	3.5	7.4	ND[a]
(1:10)[b]					
ABT-751 sulfate validation					
20	20	98.6	6.7	4.6	ND[a]
60	20	105.8	3.4	4.2	113.2
300	20	101.6	3.4	–[c]	ND[a]
4000	19	97.3	3.8	4.0	109.0
20000	20	105.5	3.3	3.9	ND[a]
(1:10)[b]					
ABT-751 in-study					
60	13	99.4	5.6	3.1	ND[a]
300	13	98.0	5.1	4.8	ND[a]
4000	13	98.9	7.6	–[c]	ND[a]
20000	13	103.1	5.9	3.3	ND[a]
(1:10)[b]					
ABT-751 glucuronide in-study					
60	13	97.1	5.6	2.8	ND[a]
300	13	94.2	5.9	3.9	ND[a]
4000	13	94.4	4.8	5.5	ND[a]
20000	13	98.3	5.0	4.4	ND[a]
(1:10)[b]					
ABT-751 sulfate in-study					
60	13	99.3	6.3	5.0	ND[a]
300	13	97.2	7.5	4.6	ND[a]
4000	13	94.2	13.7	–[c]	ND[a]
20000	13	101.2	5.5	5.6	ND[a]
(1:10)[b]					

[a] ND, not done.
[b] Sample diluted 1:10 (v/v) prior to analysis.
[c] No significant additional variation was observed as a result of performing the assay in different runs.

monohydrate, croscarmellose sodium and magnesium stearate and stored at room temperature. The protocol was approved by the Institutional Review Board of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (Baltimore, MD, USA), and the patient provided written informed consent.

Blood samples were collected in EDTA-containing tubes before drug administration and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 24 h after administration of the ABT-751 after 1 week of continuous administration. Samples were processed immediately by centrifugation for 10 min at 1000 × *g* at 4 °C. Plasma supernatant was stored at −70 °C until subsequent analysis. Samples were thawed and processed as described in Section 2.3.

ABT-751, ABT-751 glucuronide, and ABT-751 sulfate pharmacokinetic parameters were determined by standard non-compartmental methods using steady-state calculations in the program WinNonlin version 3.1 (Pharsight Corporation, Mountain View, CA). The C_{max} and T_{max} after oral administration were obtained by visual inspection of the plasma concentration–time curve. The area under the plasma concentration–time curve (AUC) during the dose interval ($AUC_{[0-\tau]}$) was calculated using the linear up/log down trapezoidal method. Apparent systemic clearance at steady-state (Cl_{ss}/F) was calculated by dividing the dose by $AUC_{[0-\tau]}$ for ABT-751. The terminal rate constant, λ_z, was determined from the slope of the terminal phase of the plasma concentration–time curve. The terminal half-life ($T_{1/2}$) was calculated as 0.693 divided by λ_z.

3. Results and discussion

3.1. Chromatographic separation and detection

A LC/MS/MS method to quantitatively determine ABT-751, ABT-751 glucuronide, and ABT-751 sulfate concentrations in human plasma was developed, validated, and implemented to quantitate drug in plasma from patients receiving treatment with ABT-751. The mass spectrum of ABT-751, ABT-751 glucuronide, and ABT-751 sulfate showed protonated molecules ([M + H]) at *m/z* 372.1, 548.2, and 452.0, respectively. The collision energy fragmented ABT-751 and its metabolites into several fragments. The major fragments observed were at *m/z* 200.0, 199.8, and 199.8 for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate, respectively, and were selected for subsequent monitoring in the third quadrupole (Fig. 1). The internal standard, A-93387, had a full-scan product ion spectrum of *m/z* 500.3 showing a major fragment at *m/z* 272.0 (data not shown).

No peaks were observed in the chromatograms of blank plasma from six donors when monitored for ABT-751, ABT-751 glucuronide, ABT-751 sulfate, and the internal standard (data not shown). During implementation of this assay, pre-treatment plasma samples from nine cancer patients were analyzed with this assay with no interferences noted. When a neat solution of ABT-751 sulfate was analyzed, the area count of ABT-751 was approximately 20% of ABT-751 sulfate. This value was 2% for ABT-751 glucuronide. However, due to the response of the metabolites compared to the metabolites, this does not translate into a significant concentration difference in plasma. When a plasma sample of ABT-751 sulfate or ABT-751 glucuronide alone was analyzed, the concentration of ABT-751 that was formed was 1.3% for ABT-751 sulfate and 0.9% for ABT-751 glucuronide. Representative chromatograms of blank human plasma, plasma spiked with ABT-751, ABT-751 glucuronide, and ABT-751 sulfate, and a patient plasma sample (8 h time point) from a patient that received 150 mg of ABT-751 administered orally are shown in Fig. 2. The retention times for ABT-751, ABT-751 glucuronide, ABT-751 sulfate, and the internal standard under the optimal conditions were 1.30, 1.20, 1.30, and 3.50 min, respectively. The overall chromatographic run time was 5 min.

Table 4
Assessment of stability

Nominal concentration (ng/ml)	Stability (% of initial)					
	Freeze-thaw cycles[a]			Autosampler stability[b]	Long-term stability (−70 °C)[a]	
	1	2	3		11 days	109 days
ABT-751						
60	100.7	101.1	89.2	99.8	100.8	100.9
4000	101.4	107.4	106.1	101.8	114.9	104.5
20000 (1:10)[c]	95.2	97.8	103.1	–[d]	96.8	104.8
ABT-751 glucuronide						
60	97.7	103.4	103.9	97.0	93.1	82.9
4000	102.1	102.0	104.7	96.8	97.0	79.3
20000 (1:10)[c]	102.2	108.8	110.6	–[d]	86.7	88.1
ABT-751 sulfate						
60	100.5	101.6	110.0	103.7	96.5	79.7
4000	105.2	109.4	109.8	100.3	98.8	83.4
20000 (1:10)[c]	103.8	109.3	107.4	–[d]	90.9	84.8

[a] Performed in triplicate in plasma.
[b] Performed repeatedly for 9.8 h with one sample that was extracted from plasma.
[c] Sample diluted 1:10 (v/v) with plasma prior to analysis.
[d] Not done.

3.2. Linearity of detector responses

Using a quadratic standard curve over the entire range of 20–5000 ng/ml with a weighting factor (1/[nominal concentration]), calibration curves for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate standards were constructed from the peak area ratio of the analyte to the internal standard with an correlation coefficient ($r > 0.99$, range = 0.9979–0.9996). The weighting factor was chosen compared to uniform weighting after evaluation of goodness-of-fit by assessment of the correlation coefficient, intercept closest to a zero value, % recovery of calibrators and QC samples, and assessment of residuals.

For each point on the calibration curves for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate, the concentrations back-calculated from the equation of the regression analysis were always within 8.0% of the nominal value (Table 2). A linear regression of the back-calculated concentrations versus the nominal values provided a unit slope and an intercept not significantly different from zero (data not shown). The mean ± standard deviation of the equation was 0.0566 ± 0.0318 for the a_0 parameter, 0.0171 ± 0.0022 for the a_1 parameter, and $-1.0835 \times 10^{-6} \pm 0.2173 \times 10^{-6}$ for the a_2 parameter ($n = 4$) were calculated for the ABT-751 calibration curve. For the ABT-751 glucuronide calibration curve, the equation was 0.0020 ± 0.0047 for the a_0 parameter, 0.0018 ± 0.0003 for the a_1 parameter, and $-7.7100 \times 10^{-8} \pm 0.8787 \times 10^{-8}$ for the a_2 parameter ($n = 4$). The equation was 0.0040 ± 0.0036 for the a_0 parameter, 0.0024 ± 0.0005 for the a_1 parameter, and $-1.2350 \times 10^{-7} \pm 0.1555 \times 10^{-7}$ for the a_2 parameter ($n = 4$) were calculated for the ABT-751 sulfate calibration curve. The distribution of the residuals showed random variation, was normally distributed, and centered on zero (data not shown).

The LLOQ for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate was established at 20 ng/ml for human plasma, at which the concentration was associated with a mean ± standard deviation signal-to-noise ratio of 2785 ± 1271; 540 ± 282; and 1001 ± 472 from 20 observations.

3.3. Accuracy, precision, and recovery

For QC samples prepared by spiking human plasma with ABT-751, ABT-751 glucuronide, and ABT-751 sulfate, the within-run and between-run variability (precision), expressed as the percentage relative standard deviations, was less than or equal to 8.3%. The mean predicted concentration (accuracy) was less than 6.4% of the nominal value for the QC samples (Table 3). During in-study validation, the within-run and between-run variability was less than 13.7% and accuracy was less that 5.8% of the nominal value. The relative recovery of ABT-751 varied from 90.3% to 100.8% for 60 and 4000 ng/ml, respectively (Table 3). For ABT-751 sulfate, the relative recovery varied from 109.0% to 113.2%. The relative recovery for ABT-751 glucuronide (29.6–30.6%) was lower than ABT-751 and ABT-751 sulfate but consistent over the concentration range.

Table 5
Cross-validation of ABT-751 analytical methods[a]

Sample ID	ABT-751 (ng/ml)			ABT-751 glucuronide (ng/ml)			ABT-751 sulfate (ng/ml)		
	Theoretical concentration	Abbott results	Hopkins results	Theoretical concentration	Abbott results	Hopkins results	Theoretical concentration	Abbott results	Hopkins results
Pre	0.0	BLQ[b]	BLQ	0.0	BLQ	BLQ	0.0	BLQ	BLQ
15 min	502.0	463.6 (92.4%)	483.9 (96.4%)	21.7	22.9 (105.5%)	26.6 (122.6%)	84.7	77.4 (91.4%)	128.5 (151.7%)
30 min	2379.5	2678.2 (112.6%)	2391.3 (100.5%)	188.4	193.2 (102.5%)	193.4 (102.7%)	426.3	452.0 (106.0%)	543.5 (127.5%)
1 h	6437.6	6490.4 (100.8%)	5800.0 (90.1%)	1089.4	1160.8 (106.6%)	1000.8 (91.9%)	2079.8	2206.6 (106.1%)	2310.8 (111.1%)
2 h	5432.1	6014.1 (110.7%)	4837.5 (89.1%)	2271.6	2575.5 (113.4%)	2168.8 (95.5%)	4642.0	5062.0 (109.0%)	5327.5 (114.8%)
4 h	3753.5	3887.7 (103.6%)	3811.3 (101.5%)	3062.0	3099.5 (101.2%)	3283.8 (107.2%)	5432.6	5052.4 (93.0%)	6200.0 (114.1%)
6 h	1979.6	2290.4 (115.7%)	1946.7 (98.3%)	3167.4	3734.8 (117.9%)	3579.2 (113.0%)	5048.0	6274.0 (124.3%)	5933.3 (117.5%)
9 h	1389.0	1571.6 (113.1%)	1367.5 (98.5%)	2579.6	3008.3 (116.6%)	2946.7 (114.2%)	3869.4	4432.7 (114.6%)	4658.8 (120.4%)
24 h	288.3	267.7 (92.9%)	280.1 (97.2%)	1193.0	1280.0 (107.3%)	1378.3 (115.5%)	1789.4	1888.8 (105.6%)	2417.5 (135.1%)

[a] Results are reported in concentration values and as the percentage of the theoretical concentration in parenthesis.
[b] BLQ, below limits of quantitation.

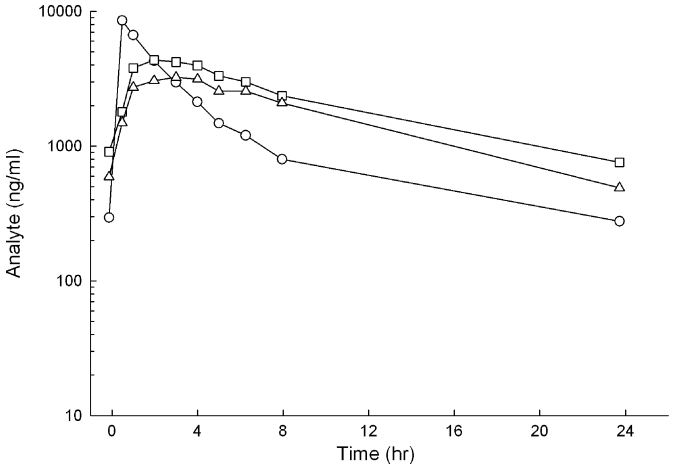

Fig. 3. Plasma concentration–time curve in a single patient for ABT-751 administered orally at a dose of 150 mg. The open circle (◯) represents ABT-751 concentrations, the open triangle (△) represents ABT-751 glucuronide concentrations, and the open square (□) represents ABT-751 sulfate concentrations.

3.4. Analyte stability

ABT-751, ABT-751 glucuronide, ABT-751 sulfate, and the internal standard were stable on the auto-injector at room temperature for 84 injections (9.83 h) when protected from light with aluminum foil covering the autosampler (see Table 4). ABT-751, ABT-751 glucuronide, and ABT-751 sulfate were stable after 3 freeze-thaw cycles at −70 °C (see Table 4). Long-term stability studies from 109 days demonstrate ABT-751 is stable but there is a slight decrease in ABT-751 glucuronide and ABT-751 sulfate at 109 days (see Table 4).

3.5. Cross-validation samples

This LC/MS/MS method was applied to the quantitation of ABT-751 and metabolites spiked in plasma samples from Abbott. The results from both analytical methodologies are reported in Table 5. Both analytical methods result in similar concentrations without bias.

3.6. Plasma concentration–time profile

This LC/MS/MS method was applied to the quantitation of ABT-751 and metabolites in plasma samples from a patient who has received ABT-751 administered alone at a dose of 150 mg. A single patient concentration versus time profile is illustrated in Fig. 3 after 1 week of oral ABT-751. This patient had a C_{max} of 8540.0 ng/ml for ABT-751, 3240.0 ng/ml for ABT-751 glucuronide, 4370.0 ng/ml for ABT-751 sulfate that occurred at 0.5, 3.0, and 2.0 h, respectively. The $T_{1/2}$ was 9.0 h for ABT-751, 7.4 h for ABT-751 glucuronide, and 9.1 h for ABT-751 sulfate. The Cl_{ss}/F for ABT-751 was 4.9 l/h.

4. Conclusions

We have developed and validated a LC/MS/MS assay for measuring ABT-751 and its metabolites (ABT-751 glucuronide and ABT-751 sulfate) concentrations in human plasma under current requirements as to validation of bioanalytical methodologies [8]. The described method permits the analysis of patient plasma samples for ABT-751, ABT-751 glucuronide, and ABT-751 sulfate, which is sufficiently sensitive to allow pharmacokinetic monitoring after oral administration of ABT-751 at doses of 150 mg. This LC/MS/MS method will be used in the ongoing Phase I trial in colorectal cancer patients to measure ABT-751 and metabolite concentrations in human plasma to fully characterize the clinical pharmacology of this agent.

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References

[1] K. Yoshimatsu, A. Yamaguchi, H. Yoshino, N. Koyanagi, K. Kitoh, Cancer Res. 57 (1997) 3208–3213.
[2] C.M. Galmarini, Curr. Opin. Investig. Drugs 6 (2005) 623–630.
[3] N. Koyanagi, T. Nagasu, F. Fujita, T. Watanabe, K. Tsukahara, Y. Funahashi, M. Fujita, T. Taguchi, H. Yoshino, K. Kitoh, Cancer Res. 54 (1994) 1702–1706.
[4] K. Yamamoto, K. Noda, A. Yoshimura, M. Fukuoka, K. Furuse, H. Niitani, Cancer Chemother. Pharmacol. 42 (1998) 127–134.
[5] S.Y. Cho, P.C. Adamson, A.E. Hagey, B.C. Widemann, J.M. Maris, E. Fox, D. Medina, Y. Cui, G.B. Gordon, F.M. Balis, Proc. Am. Soc. Clin. Oncol. 23 (2004) 147 (abstract 2080).
[6] K.R. Hande, K. Meek, A.C. Lockhart, S. Glad-Anderson, J. Berlin, H. Eliopoulos, B. Roth, P. Roberts, M.L. Rothenberg, Proc. Am. Soc. Clin. Oncol. 22 (2003) 130 (abstract 520).
[7] D.J. Daszkowski, Abbott Laboratories R&D/01/616. Internal Abbott Document. 10 pp.
[8] C. Hartmann, J. Smeyers-Verbeke, D.L. Massart, R.D. McDowall, J. Pharm. Biomed. Anal. 17 (1998) 193–218.