DMD Fast Forward. Published on October 2, 2008 as doi:10.1124/dmd.108.023143

DMD #23143

APIXABAN METABOLISM AND PHARMACOKINETICS FOLLOWING ORAL ADMINISTRATION TO HUMANS

Nirmala Raghavan, Charles E Frost, Zhigang Yu, Kan He, Haiying Zhang, W Griffith Humphreys, Donald Pinto, Shiangyuan Chen, Samuel Bonacorsi, Pancras C Wong, Donglu Zhang

Pharmaceutical Candidate Optimization (NR, KH, HZ, WGH, and DZ), Discovery Medicine and Clinical Pharmacology (CF, ZY), Discovery Chemistry and Biology (DP, SC, SB, PCW), Bristol-Myers Squibb Research and Development, Princeton, New Jersey

Running title: Metabolism and pharmacokinetics of apixaban in humans

Please forward all correspondence to:
Dr. Donglu Zhang
PO Box 4000
Pharmaceutical Candidate Optimization
Bristol-Myers Squibb Research and Development
Princeton, New Jersey 08543-4000
Tel: +1 609 252 5582
Fax: +1 609 252 6802
Email: donglu.zhang@bms.com

Abbreviations: ADME, absorption, distribution, metabolism, and elimination; AUC, The area under the plasma concentration vs. time curve; BID, twice daily dose; DPM, disintegrations per minute; LC/MS, liquid chromatography/mass spectrometry; HPLC, high pressure liquid chromatography; HRMS, high resolution mass spectrometry; NMR, Nuclear magnetic resonance; PK, pharmacokinetics; TRA, total radioactivity

Word count in Abstract: 220 (max 250)
Word in Introduction: 630
Word in Discussion: 650
Number of tables/figures: 4/4
Number of references: 30

ABSTRACT

The metabolism and disposition of [^{14}C]apixaban, an orally bioavailable, highly selective, and direct acting/reversible factor Xa inhibitor, was investigated in 10 healthy male subjects without (Group 1, n=6) and with bile collection (Group 2, n=4) after a single 20 mg oral dose. Urine, blood and feces samples were collected from all subjects. Bile samples were also collected for 3–8 hours after dosing from Group 2 subjects. There were no serious adverse events (AEs) or discontinuations due to adverse effects. In plasma, apixaban was the major circulating component and *O*-demethyl apixaban sulfate, a stable and water soluble metabolite, was the significant metabolite. The exposure of apixaban (C_{max} and AUC) in subjects with bile collection was generally similar to subjects without bile collection. The administered dose was recovered in feces (Group 1, 56.0%; Group 2, 46.7%) and urine (Group 1, 24.5%; Group 2, 28.8%), with the parent drug representing approximately half of the recovered dose. Biliary excretion represented a minor elimination pathway (2.44% of the administered dose) from Group 2 subjects within the limited collection period. Metabolic pathways identified for apixaban included *O*-demethylation, hydroxylation and sulfation of hydroxylated *O*-demethyl apixaban. Thus, apixaban is an orally bioavailable inhibitor of FXa with elimination pathways that include metabolism and renal excretion.

INTRODUCTION

Thromboembolic events, including acute myocardial infarction, unstable angina, deep vein thrombosis (DVT), pulmonary embolism (PE) and ischemic stroke, continue to be the leading cause of morbidity and mortality in the USA and other Western countries (Heit et al., 2005; Rosamond et al., 2007). Current therapies for the treatment and prevention of thromboembolic events, such as vitamin K antagonists (e.g., warfarin), heparin and low molecular weight heparin (e.g., enoxaparin), are suboptimal (O'Donnell and Weitz, 2004; Wittkowsky, 2004; Campbell, 2006; GlaxoSmithKline, 2007). However, the requirement for intravenous or subcutaneous injection and/or the need for careful monitoring due to the risk of excessive bleeding or unpredictable/inconsistent pharmacokinetics (PK) can complicate administration and present barriers to the use of these agents (O'Brien and Caro, 2002; Wittkowsky, 2004; Campbell, 2006; GlaxoSmithKline, 2007). Therefore, new, orally active anticoagulants with predictable PK profiles are needed that can be administered with a reduced need for monitoring.

Factor Xa is a key serine protease in the coagulation cascade and is a promising target enzyme for new therapeutic agents for the treatment and prevention of arterial and venous thrombosis (Kaiser, 2002; Samama, 2002; Walenga et al., 2003). Specifically, factor Xa plays a critical role in blood coagulation, serving as the juncture between the extrinsic (tissue factor initiated) and intrinsic (surface activation and amplification) systems (Mann et al., 2003). Factor Xa forms the prothrombinase complex with phospholipids, calcium ions and a co-factor, factor Va, which is responsible for the generation of thrombin from prothrombin. Although factor Xa inhibition attenuates the generation of thrombin, it does not affect thrombin activity, thereby preserving hemostasis, which, in clinical terms, may translate to efficacy with

lower bleeding risk (Comp, 2003; Kubitza and Haas, 2006). Clinical proof-of-principle for the efficacy and potential of factor Xa inhibitors is available from fondaparinux, an indirect factor Xa inhibitor (Walenga et al., 1988; Samama and Gerotziafas, 2003; Simoons et al., 2004; Wong et al., 2006; Yusuf et al., 2006; Yusuf et al., 2006; Schumacher et al., 2007).

Apixaban is an orally bioavailable, highly selective, direct acting/reversible factor Xa inhibitor in late-stage clinical development for the prevention and treatment of thromboembolic diseases. Apixaban has a high affinity for human factor Xa with a relatively low affinity for thrombin and trypsin; the high affinity and selectivity of apixaban may translate into improved pharmacologic characteristics versus preceding oral factor Xa compounds leading to an improved treatment option (Pinto et al., 2007; Wong et al., 2008). Positive results from a Phase II clinical trial evaluating the prevention of venous thromboembolic events (VTE, asymptomatic and symptomatic DVT and non-fatal symptomatic PE) demonstrate the potential benefit of this agent versus standard therapy in anticoagulation monotherapy treatment (Lassen et al., 2007; Buller et al., 2008). Apixaban demonstrated a linear pharmacokinetics in the range of 2.5 to 25 mg oral doses in humans (Frost et al., 2007a). Here, we evaluated the metabolism and PK of apixaban following oral administration in healthy volunteers.

METHODS

Study design

This was an open-label single-dose study in healthy male subjects 18 to 45 years of age. All subjects were in good health as determined by medical history, physical examination, vital sign and electrocardiogram assessment and clinical laboratory tests. Subjects with a previous medical history of coagulopathy or adverse reaction

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

to anticoagulant or antiplatelet agents were excluded. Subjects who met all inclusion and exclusion criteria and provided informed written consent were admitted to the clinical facility. Subjects were assigned to 1 of 2 groups, Group 1 (n=6) or Group 2 (n=4). In addition to other study procedures, bile samples were collected from subjects in Group 2. After ≥10-hour overnight fast, each subject received a single dose of 20 mg [^{14}C]apixaban containing 108.8 μCi of radioactivity as an oral solution in polyethylene glycol-400/ethanol (85:15, v/v). All subjects remained in the clinical facility for 12 days. Urine and fecal samples were collected continuously over 24-hour intervals upto 240 hour after or until radioactivity in the sample was ≤1% the administered dose. Bile samples were collected by continuous suction through a nasogastric tube at 3–4, 4–6 and 6–8 hours postdose from subjects in Group 2. Blood samples were collected at 1, 6, 12, 24, 48 and 96 hours postdose in all subjects for biotransformation analysis. Blood samples for measuring apixaban and radioactivity were collected at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 36, 48, 96, 120, 144, 168, and 192 h postdose. Subjects were discharged from the clinic in the afternoon of Day 13 provided that the Day 7 measurement of radioactivity in feces was ≤1% of administered radioactivity. To ensure defecation prior to discharge a 30 mL dose of Milk of Magnesia was administered on Day 6. All subjects were closely monitored for adverse events throughout the study.

The study was performed in accordance with local regulations and the principles of the Declaration of Helsinki, its amendments, and Good Clinical Practice. The protocol and informed consent were approved by the New England Institutional Review Board (Wellesley, MA). The clinical phase of the study was conducted at Clinical Applications Laboratories Inc., (San Diego, CA).

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Radiolabeled drug and chemicals

Apixaban, 1-(4-methoxyphenyl)-7-oxo-6-[4-(2-oxopiperidin-1-yl)phenyl]-4,5-dihydro pyrazolo[5,4-c]pyridine-3-carboxamide (BMS-562247, purity 99.1%), and [^{14}C]apixaban were synthesized at Bristol-Myers Squibb (Princeton, NJ). The specific radioactivity of [^{14}C]apixaban was 5.44 μCi/mg (98.6% radiochemical purity).

Formic acid and ammonium hydroxide were purchased from Fischer Scientific Co. (Fair Lawn, NJ). Deepwell LumaPlate^{TM}-96-well plates were purchased from PerkinElmer^{TM} (Boston, MA) and Ecolite^{TM} liquid scintillation cocktail was purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). All other organic solvents and reagents were of high performance liquid chromatography (HPLC) grade.

Synthesis of metabolite standards

Preparation of 1-(4-hydroxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (*O*-demethyl apixaban)

Apixaban (4.4 g, 9.5 mmol) was mixed with pyridine hydrochloride (6.6 g, 57 mmol) and heated at 200^{0}C for 5 hours. The reaction was cooled and solid materials were suspended in a mixture of CH_2Cl_2/water (1:1, v/v, 100 mL). Sodium bicarbonate (10 g) was added to this suspension and the insoluble solid precipitate was filtered to afford 7.3 g white powder. Approximately 4 g of this solid powder was subjected to purification via silica gel column chromatography (methanol/CH_2Cl_2, 1:9, v/v) to afford 0.76 g of the phenol product. The balance of the crude phenol powder was suspended in aqueous NH_4OH and washed with dilute acid (1N HCl, 100 mL), dried ($MgSO_4$) and purified by chromatography as described above to afford additional phenol product (0.117 g). The overall yield for *O*-demethyl apixaban was 30.9%. ^{1}H NMR ($CDCl_3$) δ (PPM): 7.34 (2H, d, J = 8.8Hz), 7.33 (2H, d, J = 8.8Hz), 7.25 (2H, d,

J = 8.8Hz), 6.80 (1H, s), 6.76 (2H, d, J = 8.8Hz), 6.35 (1H, s), 5.44 (1H, s), 4.10 (2H, t, J = 6.6Hz), 5.87 (2H, t, J = 5.87Hz), 3.38 (2H, t, J = 6.6Hz), 2.57 (2H, t, J = 5.38Hz), 1.94 (4H, m); HRMS calc'd $[M+H]^+$ for $C_{24}H_{24}N_5O_4$ 446.1828; found $[M+H]^+$ 446.1817.

Preparation of 4-(3-carbamoyl-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetra-hydropyrazolo[3,4-c]pyridin-1-yl)phenyl hydrogen sulfate (O-demethyl apixaban sulfate)

To *O*-demethyl apixaban (0.48 g,1.07 mmol) in pyridine (2 mL), was added sulfur trioxide in triethylamine (0.45 g, 3.2 mmol) and the reaction was stirred at room temperature for 72 hours. An additional (0.1 g, 0.71 mmol) of sulfurtrioxide-triethylamine mixture was added and the reaction was stirred for an additional 24 hours. The reaction was diluted with diethyl ether and the precipitated solid was filtered off. The filtrate was dissolved in water (50 mL) and the pH was adjusted with 1N NaOH until pH = 8. The aqueous solution was than extracted with CH_2Cl_2 (2 X 25 mL) to remove unreacted phenol. The pH was again adjusted with 1N HCl (pH = 5) and this solution was used directly onto a reverse phase HPLC system using water/CH_3CN with 0.05% TFA for purification. The isolated product fractions were concentrated *in vacuo* and freeze-dried. Analysis of the recovered solid showed some phenol *O*-demethyl apixaban present. The sample was again re-purified via reverse phase HPLC using water with 0.01M ammonium acetate buffer and CH_3CN. The pure fractions were concentrated with a stream of N_2 and freeze-dried to afford 231 mg (39%) of the ammonium salt of *O*-demethyl apixaban sulfate (purity 90.0%). ^{1}H NMR DMSO-d6 δ (ppm): 7.74 (1H, s), 7.49 (2H, d, J = 8.8Hz), 7.42 (1H, s), 7.38 (2H, d, J = 8.8Hz), 7.29 (2H, d, J = 8.8Hz), 7.24 (2H, d, J = 8.8Hz), 7.09 (5H, br s),

4.08 (2H, t, J = 6.6Hz), 3.62 (2H, t, J = 5.6Hz), 3.23 (2H, t, J = 6.6Hz), 2.41 (2H, t, J = 6.1Hz), 1.87 (4H, m). HRMS Calc'd $C_{24}H_{24}N_5O_7S$ $[M+H]^+$ 526.1396 found $[M+H]^+$ 526.1379.

Collection and preparation of blood, bile, urine and feces samples

Blood samples

Blood samples were collected via an indwelling catheter or by direct venipuncture using vacutainers containing K_2EDTA as coagulant, and immediately placed on ice. Duplicate gravimetric aliquots (~100 mg) of whole blood samples were combusted in a sample oxidizer prior to scintillation counting for total radioactivity (TRA). The remaining blood sample was centrifuged to harvest plasma. All plasma samples were frozen and stored at –20^{o}C. Duplicate gravimetric aliquots (~100 mg) of each plasma sample were analyzed for TRA.

Pooled plasma samples (at 1, 6, 12, 24, 48 and 96 hours) from each group were prepared separately by mixing an equal volume (0.5 mL) of plasma sample from each subject. Each pooled plasma sample was extracted in duplicate at each time point by addition of 4 mL of acetonitrile/methanol (1:1, v/v) to 1 mL of plasma while the sample was mixed on a vortex mixer; each supernatant fraction was removed and saved. The precipitate was then re-suspended in 2 mL acetonitrile and 1 mL methanol. After centrifugation the supernatant fraction was removed and combined with the first supernatant. The final precipitate was re-suspended in 2 mL acetonitrile. After centrifugation the supernatant fraction was removed and combined with the first and second supernatants. Extraction recovery of radioactivity (percent of sample) from pooled human plasma after an oral dose of [^{14}C]apixaban was calculated as radioactivity in the extract divided by radioactivity in the sample. The average extraction recovery from the replicate (n=2) plasma samples was 85-95% for all time

points. The combined supernatant fraction was evaporated to dryness under nitrogen and reconstituted in 0.15 mL of acetonitrile and 0.05 mL of methanol. After centrifugation, a 0.06–0.1 mL portion of supernatant was injected into the HPLC.

Bile samples

Bile samples were collected from Group 2 subjects using a weighted oral-gastro-duodenal tube and aspiration methods described previously (Wang et al., 2006; Strasberg et al., 1990; Choudhuri et al., 1993). One hour after drug administration, suction ports at the terminal end of an oral-gastro-duodenal tube were positioned at the vertical limb of the duodenal loop, near the ampulla of Vater (confirmed via fluoroscopy). Suction ports remained in the gastric antrium of the stomach to collect gastric juice in a separate inner lumen. At 7 hours post dose, a 20 ng/kg intravenous dose of cholecystokinin carboxyl-terminal octapeptide (Kinevac®) was infused over 5 minutes to stimulate gallbladder contraction and enhance bile flow. Bile was collected for 3-8 hours postdose. The bile samples were immediately frozen and stored at –70^{o}C. Prior to analysis, the bile was thawed at room temperature and diluted with water (1:1). Portions of 0.1 mL were injected into the HLPC or ion trap analysis and 0.03 mL was used for quadrapole time of flight (Q-TOF) mass spectrometry (MS) analysis.

Urine and fecal samples

All urine and feces samples were collected, frozen and stored at –20^{o}C. Pooled urine samples were prepared by combining 10% of each collection by volume from all subjects from each group. Prior to analysis, the urine was thawed at room temperature. Centrifugation was then performed and 0.1 mL of urine was injected into the HLPC. Water/reagent ethanol (50:50, v/v) was added to each fecal sample to form an approximate 20% (w/w) feces/solvent mixture, which was homogenized

using a probe-type homogenizer. Fecal homogenates were stored at –20^{o}C. Pooled feces sample were prepared by combining 5% by weight from all collections from each subject of each group. Each pooled fecal homogenate sample (0.5 mL) was extracted in replicates by addition of 1.5 mL of methanol/acetonitrile (1:2, v/v) while the sample was mixed on a vortex mixer. The extraction was repeated and the supernatants were combined. Extraction recovery of radioactivity (percent of sample) from pooled human feces was calculated as radioactivity in the extract divided by radioactivity in the sample. The average extraction recovery from the replicate (n=4) fecal samples was 86-93%.The supernatant fraction, following two extractions, was evaporated to dryness under nitrogen and reconstituted in 0.15 mL of methanol. After centrifugation, a 0.04 mL portion of supernatant was injected into the HPLC.

Measurement of radioactivity

The levels of total radioactivity in blood, plasma, urine, bile and feces were determined by liquid scintillation counting (LSC) after an aliquot of each sample was combusted. Sample combustion was performed with a sample oxidizer (PerkinElmer Life Sciences, Inc., Boston, MA). The resulting $^{14}CO_2$ was trapped with Carbo-Sorb E (PerkinElmer Life Sciences, Inc.), mixed with Permafluor E+ (PerkinElmer Life Sciences, Inc.) scintillation fluid, and the radioactivity was quantified over 5 minutes using LSC. Liquid scintillation counter data were automatically corrected for counting efficiency using an external standardization technique and an instrument-stored quench curve generated from a series of sealed quench standards.

The combustion efficiency was determined prior to combustion of experimental samples using a commercial ^{14}C standard. Specifically, ^{14}C-fortified scintillation fluid or fecal homogenate samples fortified with the same amount of radioactivity at three levels of radioactivity (low, medium and high standards) were combusted and then

counted. The average DPM recovered after combustion was then compared in the scintillation fluid or the fecal homogenate to determine the combustion efficiency value. Combustion efficiency was within 100% ± 5% (100% ± 10% for feces).

The lower limit of quantitation of radioactivity in blood and plasma under these conditions was 45.0-53.4 and 29.2-31.7 ng-apixaban equivalents/mL, respectively. The lower limit of detection of radioactivity in urine, bile and fecal homogenate under these conditions was 20–30 dpm. The volume of bile and urine, and weight of feces collected over each interval and the concentrations of radioactivity in the corresponding samples were used to calculate the cumulative percent of the administered dose recovered in the bile, urine and feces.

Measurement of apixaban concentrations

The concentrations of apixaban in plasma were determined by a validated LC/MS/MS method using 0.2 mL of plasma and $^{13}CD_3$ - labeled apixaban as the internal standard. The low limit of quantitation of apixaban in plasma under these conditions was 1 ng/mL. After solid extraction on a 3M empore C8-SD 96-well solid extraction plate, HPLC on a Shimadzu LC-10AT system (Shimadzu Scientific Instruments, Kyoto, Japan) was performed isocratically on a Phenomenex Luna C18(2) analytical column (2.1 x 50 mm, 5 μ). The mobile phase contains 0.1% formic acid in acetonitrile and water (60:40, v/v) at 0.3 ml/min. The mass analysis was performed on a Sciex API3000 mass spectrometer (PE-Sciex Concord, Ontario, Canada). The detection was by positive ion electrospray tandem mass spectrometry with the mass transition of M/Z 460 to 443.

Stability testing of M1

Synthetic metabolite M1 was fortified in human plasma at 10 and 500 ng/ml concentrations in triplicate. The metabolite was extracted from 100 μl of plasma by

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

protein precipitation using 300 μL of acetonitrile and methanol (3:1, v/v). The supernatant was then diluted and analyzed by LC/MS/MS with a mass transition of 526.2 to 446.1 in a similar manner to analysis of apixaban. Concentration standards were prepared similarly at 5, 10, 50, 100, 500, 800, and 1000 ng/ml of synthetic M1. The stable isotope-labeled apixaban was used as the internal standard.

Pharmacokinetic analysis

The mean plasma concentration versus time data for radioactivity and unchanged apixaban were analyzed with a noncompartmental method (Perrier and Gibaldi, 1982). The peak plasma concentration (C_{max}) and the time to reach peak concentration (T_{max}) were recorded directly from experimental observations. Total radioactivity was converted to apixaban ng-equivalents based on radioactive specific activity of [^{14}C]apixaban (5.44 μCi/mg). The area under the plasma concentration versus time curve from 0 to T (AUC_{0-T}), where T equals the time of the last measured plasma concentration, was calculated by a combination of conventional trapezoidal and log-trapezoidal methods by Kinetica (version 4.4). AUC to infinity ($AUC_{0-\infty}$) as determined by the sum of AUC_{0-T} and the extrapolated area. The extrapolated area was determined by dividing the last measured concentration by the slope of the terminal log-linear phase. The terminal slope (λ) of the plasma concentration-time profile was determined by the method of least squares (log-linear regression of at least three data points). The terminal half-life was estimated as $\ln 2/\lambda$.

Estimation of metabolite exposure was obtained from the relative distribution of metabolite in the plasma, the specific activity (5.44 μCi/mg) of the administered apixaban, and the total concentration of radioactivity in plasma sample obtained for biotransformation analysis at limited time points (1, 6, 12, 24 and 48 hours).

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Metabolite profiles

Sample analysis by HPLC was performed on a Shimadzu LC-10AT system (Shimadzu Scientific Instruments, Kyoto, Japan) equipped with a photodiode array ultraviolet (UV) detector. An Ace 3, C18 (3 μm), 150 x 4.6 mm column (MacMod, Chaddsford, PA) was used, with a mobile phase flow rate of 0.7 mL/min. An Ace 3, C18 (3 μm), 150 x 2.1 mm column (MacMod, Chaddsford, PA) was used for Q-TOF MS analysis, with a mobile phase flow rate of 0.17 mL/min. The retention times of reference standards were confirmed by their UV spectra. The HPLC solvent system was a gradient of two solvents of 0.4% formic acid in water, pH 3.2 (A) and 100% acetonitrile (B). The gradient for elution was 0% B for 3 min; 0-10% B in 2 min; 10-25% B in 15 min, hold 25% B for 30 min; 25-50% B in 10 min and 50-100% B in 5 min; hold 100% B for 5 min.

For quantification of radioactivity, HPLC effluent was collected in 0.26-minute intervals using a Gilson Model 204 fraction collector (Gilson Medical Electronics, Middleton, WI). The plates were dried in an automatic environmental speed vacuum and counted for radioactivity for 10 minutes (counts per minute, CPM) using a Packard TopCount NXT microplate scintillation and luminescence counter (Packard Instrument Company, Meriden, CT). Radiochromatograms were reconstructed from the radioactivity in each collection versus collection times. For each injection, the average CPM value from a baseline section of 2–3 minutes in the chromatogram was subtracted from the CPM value of each fraction.

Metabolism profiles were prepared by plotting the CPM values against time-after-injection. Radioactivity peaks in the metabolism profiles were reported as a percentage of the total radioactivity collected during the entire HPLC run.

Metabolite identification

Bile, urine and the extracts of pooled plasma and fecal samples were analyzed by LC/MS/MS using a Finnigan LTQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA). The HPLC eluate was directed to the mass spectrometer through a valve set to divert the flow from 0–5 minutes. The eluate flow was then directed to the mass spectrometer from 5 minutes until the end of the HPLC run. The capillary temperature for analysis was set to 230°C and the nitrogen gas flow rate, spray current and voltages were adjusted to give the maximum sensitivity for apixaban. Bile and plasma samples were also analyzed by high resolution mass spectral analysis using a Q-TOF/MS (Waters, Boston, MA).

RESULTS

Recovery of radioactive dose

Maximum excretion of [^{14}C]apixaban in urine and feces was from 0–24 hours and 24–48 hours, respectively, in both subject groups. The recovery of the radioactive dose in bile of Group 2 was 2.44% (0.25, 0.82, and 1.37% of dose for 3-4, 4-6, and 6-8 h collection, respectively). The excretion of radioactivity in urine and feces over 0–292-hour collection period from Group 1 was 24.5% and 56.0% of the dose, respectively, and from Group 2 over 0–240 hour collection period was 28.8% and 46.7% of the dose, respectively.

Pharmacokinetic parameters

A summary of estimated PK parameters is given in Table 1 and the plasma concentration versus time curve for apixaban and total radioactivity are shown in Figure 1. Apixaban plasma concentration-time profiles were comparable between Groups 1 and 2, indicating the bile collection procedures had minimal if any impact on apixaban PK. Apixaban was rapidly absorbed and achieved C_{max} at 1 hour on

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

average after oral dose administration. After reaching C_{max}, the apixaban plasma concentration time profile exhibited an initial rapid decline then a more gradual terminal phase, with an average $T_{1/2}$ of 12.7 h. The mean C_{max} and $AUC_{(0-\infty)}$ values for total plasma radioactivity were 509 ng-equivalent/mL and 5872 ng-equivalent•h/mL, respectively, versus 469 ng/mL and 4101 ng•h/mL, respectively, for apixaban. Apixaban plama $AUC_{(0-\infty)}$ represented approximately 70% of total radioactivity $AUC_{(0-\infty)}$ indicating that unchanged apixaban accounted for the majority of circulating radioactivity. Correspondingly, the apparent clearance of TRA represented approximately 68% of that of apixaban (Table 1). The half-life of TRA appeared shorter than that of apixaban, 8.2 h vs 12.7 h, which was likely due to differences in LLOQ. For most of subjects, plasma exposure was below TRA LLOQ within 36 h after dose administration versus 96 h for apixaban LLOQ by LC/MS/MS analysis. Mean blood to plasma radioactivity concentration ratios for both groups were relatively constant at approximately 0.71-0.83 at 0.5, 1, 1.5, 2, 4, 8, and 12 h postdose, indicating more radioactivity in plasma than in blood per unit volume. The concentration of *O*-demethyl apixaban sulfate showed a T_{max} at 6 h followed by a near mono-phasic decline that was similar to the parent.

Identification of metabolites

Based on the radiochromatographic profiles of metabolites in urine, bile, feces and plasma (Figures 2 and 3), the prominent peaks were investigated and assigned based on mass spectrometry data and comparisons to chemically synthesized reference standards. A description of the information used for the assignment of each metabolite is detailed below.

Apixaban

Apixaban showed a molecular ion $[M+H]^+$ at *m/z* 460 and fragment ions at m/z 443, 417, 282, 241, 199, and 184 in LC/MS and MS/MS analyses.

Metabolite M1

M1 showed a molecular ion $[M+H]^+$ at *m/z* 526 that was 80 amu higher than M2, suggesting a sulfate-conjugated derivative of *O*-demethylated apixaban. Fragment ions included m/z 446 and 429 (446-17). LC/MS/MS analysis indicated fragmentation of M1 was due to loss of 80 amu, and accurate mass measurement of M1 gave a molecular ion $[M+NH_4]^+$ of 543.1666, and a derived formula of $C_{24}H_{27}N_6O_7S$. The difference between the measured mass and the theoretical mass was –0.4 mDa, further supporting the conclusion that M1 was *O*-demethylated apixaban sulfate. This metabolite was subsequently synthesized, and the retention time and the fragmentation pattern of the standard matched those of M1 in humans.

Metabolite M2

M2 showed a molecular ion $[M+H]^+$ at *m/z* 446 and a major fragment ion at m/z 429. Other fragment ions of M2 included m/z 282, 240, 227, and 185. M2 was 14 amu lower than apixaban, consistent with demethylation of the methoxy moiety. The fragment at m/z 185 was consistent with 1-(4-methoxyphenyl)-4,5-ethylene-pyrazole-3-carboxamide structure, supporting the O-demethylation of apixaban. *O*-Demethyl apixaban was subsequently synthesized and the retention time and the fragmentation pattern of the standard matched those of M2 in humans.

Metabolites M4 and M7

Metabolites M4 and M7 both showed a molecular ion $[M+H]^+$ at *m/z* 476, which was 16 amu higher than apixaban. M4 and M7 had a major fragment ion at m/z 459 in the MS/MS spectrum and m/z 199 (= 185 + 14) in the MS^3 spectrum. Other fragment

ions included m/z 298, 280, 241, 199, and 184. The fragment at m/z 199 indicated that 1-(4-methoxyphenyl)-pyrazole-3-carboxamide core structure of apixaban was not the site for hydroxylation. Based on the retention times and LC/MS/MS fragmentation, M4 and M7 were proposed as two isomers of hydroxylated apixaban.

Metabolite M10

Metabolite M10 showed a molecular ion $[M+NH_4]^+$ at m/z 559. This metabolite showed a fragment ion at m/z 462 corresponding to a loss of 80+17 amu, suggesting a metabolite of the sulfate conjugate. The fragment ion at m/z 462 was 16 amu higher than M2. Other fragment ions of M10 included m/z 542, 525, 462, and 445. Accurate mass measurement of M10 gave a molecular ion of 559.1628, and a derived formula of $C_{24}H_{27}N_6O_8S$. The difference between the measured mass and the theoretical mass was –1.7 mDa. These results were consistent with a sulfate of hydroxylated *O*-demethyl apixaban.

Metabolite M13

Metabolite M13 showed a molecular ion $[M+H]^+$ at m/z 462 that was 16 amu higher than M2 by LC/MS, consistent with a hydroxylated *O*-desmethyl apixaban. The major fragment ion of M13 was at m/z 445 (M-17). Accurate mass measurement of M13 gave a molecular ion of 462.1777 and a derived formula of $C_{24}H_{24}N_5O_5$. The difference between the measured mass and the theoretical mass was –1.3 mDa, further supporting the conclusion that M13 was a hydroxylated *O*-demethyl apixaban.

Metabolism

Metabolic profiles of [^{14}C]apixaban in plasma, bile, urine, and feces

The highest level of radioactivity in plasma occurred at 1 hour after apixaban administration and decreased over time (Figure 1). The 96-hour plasma sample was not analyzed due to low level of radioactivity. The HPLC radiochromatographic

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

profiles of plasma from Groups 1 and 2 showed that M1 contributed an increasing fraction of the TRA at late time points (Figures 2a and 2b). The relative distribution of radioactive metabolites in pooled plasma after oral administration of [^{14}C]apixaban are summarized in Table 2. Apixaban and metabolite M1 were the major radioactive components in Group 1 and Group 2 plasma samples and M1 represented approximately 25% of the estimated parent $AUC_{0–48}$ (Table 3). O-Demethyl apixaban (M2), hydroxy apixaban (M7), and sulfate of hydroxylated O-demethyl apixaban (M10) were minor metabolites with relative contribution of <1% at each time point (Table 4).

Apixaban was the major component in pooled urine and feces samples from Group 1 and Group 2 subjects. Approximately 50% of the recovered oral dose was parent compound (Table 4). The prominent metabolites were M1, M2, M7 and M13 in feces of Groups 1 and 2 subjects. Metabolites M1 and M7 were present in urine samples of both Group 1 and Group 2 subjects. The HPLC radiochromatographic profiles of pooled urine, bile (Group 2 only), and feces from Group 1 and Group 2 subjects are shown in Figures 3a and 3b, respectively.

Apixaban and metabolite M1 were the major components in bile collected 3–8 hours Table 4). The HPLC radiochromatographic profile of pooled bile is shown in Figure 3b. Apixaban and the identified metabolites together accounted for 98% of the radioactivity in the human bile samples.

Safety

Apixaban was well tolerated in the this study. Four adverse events (AE) were reported by a total of 4 subjects. AEs were considered mild (eye allergy, headache, and hyperhidrosis) or moderate (dyspepsia) in intensity. Only one adverse event was considered possibly related to the study medication (moderate dyspepsia in Group 2)

and all others were considered not related or not likely to be related. All AEs resolved without treatment.

DISCUSSION

This study reports the results of a clinical study designed to determine the metabolite profiles and routes of elimination for [^{14}C]apixaban. Data showed that after oral administration of apixaban, the majority of the recovered dose was in feces (56% of the recovered dose), with urinary excretion also representing a significant elimination pathway (24.5–28.8% of the recovered dose). Based on the dose recovery in urine and the fraction of dose excreted as metabolites of apixaban in feces, at least 43-46% of radioactive dose was absorbed following oral administration of [^{14}C]apixaban in humans. Pharmacokinetic analyses demonstrate rapid absorption of apixaban following oral administration in healthy male subjects.

The ratio of apixaban AUC($_{0-\infty}$) to TRA AUC($_{0-\infty}$) in plasma was 66-72%, indicating that the parent was the major drug-related component in circulation. This was confirmed by radioactivity profiling of plasma samples, which showed that in plasma, apixaban constituted approximately 98% of total radioactivity at 1 h and >50% at all other time points. Furthermore, the similarity between the apixaban plasma concentration-time profile and the plasma radioactivity -time profile in conjunction with the rapid decline of TRA in plasma (TRA $T_{1/2}$ of 8.2 hours), suggests there were no significant long-lasting metabolites present in circulation. The PK profile of apixaban observed in the current study was consistent with previous findings (Frost et al., 2007b). Less than 3% of the dose was excreted in bile collected between 3 and 8 hours postdose which occurred when the plasma concentration of apixaban was above 200 ng/mL, suggesting that biliary excretion was a minor elimination pathway for apixaban. Of the total dose recovered in urine (24.5-28.8%), 83-88%

was apixaban and 80% was recovered within 24 hours after dosing, which paralleled the disappearance of apixaban in plasma for that period. Together, these data indicated that renal excretion of apixaban was a significant route of apixaban elimination.

Analysis of the metabolite profile of apixaban showed that parent compound is the major drug-related component in plasma, urine and feces in humans, but there are also several metabolites, of which the most prominent one was *O*-demethyl apixaban sulfate. Apixaban metabolites accounted for approximately less than 32% of the total recovered dose. Identification of these metabolites suggests that the metabolic pathways for apixaban include *O*-demethylation, hydroxylation and sulfation of hydroxylated *O*-demethyl apixaban (Figure 4). *O*-Demethyl apixaban sulfate was the prominent circulating metabolite, representing approximately 25% of the estimated parent $AUC_{0–48}$. *O*-Demethyl apixaban sulfate was tested for its ability to inhibit factor Xa, and results indicated that M1 was an inactive against the pharmaceutical target of apixaban (data not shown).

In vitro studies with liver microsomes and hepatocytes of human and animal species indicated that the primary human metabolites identified in this study were produced in vitro systems and there was no unique metabolites formed in humans (data not shown). The presence of *O*-demethyl apixaban sulfate in the human feces suggests the relative stability of this conjugate in gastrointestinal tracts during its excretion.

Multiple factors may contribute to the <100% recovery of the radioactive dose from human subjects following oral administration of [^{14}C]apixaban in this study, including loss of samples either during the collection or processing periods, or the inherent difficulty in adequately sampling of non-homogenous processed fecal samples for determination of total radioactivity. Studies in rat and dog models have indicated

good mass balance and no tissue accumulation of radioactivity (unpublished observations). The problems sometimes associated with attaining complete mass balance in human ADME studies have been documented in a recent review of the subject (Roffey et al., 2007).

The current study demonstrates that apixaban and its metabolites are excreted by multiple elimination pathways, including renal excretion and metabolism. The multiple elimination pathways suggest that patients with hepatic or renal impairment may be treated with apixaban and that the likelihood of drug–drug significant interactions may be low. Further specifically designed clinical studies are required to fully clarify whether apixaban may be a benefit in those special populations.

In conclusion, oral single dose administration of 20 mg apixaban was safe and well tolerated. Apixaban is eliminated via metabolism and renal excretion in humans. The minimal extent of elimination of radioactivity in the bile through 3-8 h collection suggests the biliary route plays a minor role in the overall disposition of apixaban.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Vijayalakshmi Pratha and the staff of Clinical Applications Laboratories for their contributions to the conduct of this study.

REFERENCES

Büller H, Deitchman D, Prins M, Segers A (2008) Efficacy and safety of the oral direct factor Xa inhibitor apixaban for symptomatic deep-vein thrombosis. The Botticelli DVT dose-ranging study. *J Thromb Haemost* **6:**1313-1318.

Campbell CL (2006). New treatment options for acute coronary syndromes. *Am J Manag Care* **12(16 Suppl)**:S435-443.

Choudhuri G, Agarwal DK, Saraswat VA, Negi TS, Saxena R and Kapoor VK (1993). Is duodenal bile representative of gallbladder bile? A comparative study. *Scand J Gastroenterol* **28(10):**920-923.

Comp PC (2003). Selective factor Xa inhibition improves efficacy of venous thromboembolism prophylaxis in orthopedic surgery. *Pharmacotherapy* **23(6):**772-787.

Eriksson BI, Borris LC, Dahl OE, Haas S, Huisman MV, Kakkar AK, Muehlhofer E, Dierig C, Misselwitz F and Kalebo P (2006). A once-daily, oral, direct Factor Xa inhibitor, rivaroxaban (BAY 59-7939), for thromboprophylaxis after total hip replacement. *Circulation* **114(22):**2374-2381.

Frost C, Yu Z, Nepal S, Mosqueda-Garcia R, Shenker A (2007a) Apixaban, an oral direct, factor Xa inhibitor: Multiple ascending-dose safety, pharmacokinetics and pharmacodynamics in healthy subjects. *J Thromb Haemost* **5 (suppl 2):** P-M-664.

Frost C, Yu Z, Nepal S, Mosqueda-Garcia R, Shenker A (2007b) Apixaban, an oral direct, factor Xa inhibitor: single-dose safety, pharmacokinetics and pharmacodynamics in healthy subjects. *J Thromb Haemost* **5 (suppl 2):** P-M-665.

GlaxoSmithKline (2007). Arixtra (fondaparinux sodium) injection: prescribing information. Accessed March 2008. Available from: http://us.gsk.com/products/assets/us_arixtra.pdf.

Heit J, Cohen A and Anderson F (2005). Estimated annual number of incident and recurrent, non-fatal and fatal venous thromboembolism (VTE) events in the US. *Blood* **106:**Abstract 910.

Kaiser B (2002). Factor Xa-a promising target for drug development. *Cell Mol Life Sci* **59(2):**189-192.

Kubitza D and Haas S (2006). Novel factor Xa inhibitors for prevention and treatment of thromboembolic diseases. *Expert Opin Investig Drugs* **15(8):**843-855.

Lassen MR, Davidson BL, Gallus A, Pineo G, Ansell J and Deitchman D (2007). The efficacy and safety of apixaban, an oral, direct factor Xa inhibitor, as thromboprophylaxis in patients following total knee replacement. *J Thromb Haemost* **5(12):**2368-2375.

Mann K, Brummel K and Butenas S (2003). What is all that thrombin for? *J Thromb Haemost* **1(7):**1504-1514.

O'Brien JA and Caro JJ (2002). Direct medical cost of managing deep vein thrombosis according to the occurrence of complications. *Pharmacoeconomics* **20(9):**603-615.

O'Donnell M and Weitz JI (2004). Novel antithrombotic therapies for the prevention of stroke in patients with atrial fibrillation. *Am J Manag Care* **10(3 Suppl):**S72-82.

Perrier D and Gibaldi M (1982). General derivation of the equation for time to reach a certain fraction of steady state. *J Pharm Sci* **71(4):**474-475.

Pinto DJ, Orwat MJ, Koch S, Rossi KA, Alexander RS, Smallwood A, Wong PC, Rendina AR, Luettgen JM, Knabb RM, He K, Xin B, Wexler RR and Lam PY (2007). Discovery of 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[3,4-c]pyridine-3-carboxamide (apixaban, BMS-562247), a highly potent, selective, efficacious, and orally bioavailable inhibitor of blood coagulation factor Xa. *J Med Chem* **50(22):**5339-5356.

Roffey SJ, Obach RS, Gedge JI and Smith DA (2007). What is the objective of the mass balance study? A retrospective analysis of data in animal and human excretion studies employing radiolabeled drugs. *Drug Metab Rev* **39(1):**17-43.

Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, Haase N, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell CJ, Roger V, Rumsfeld J, Sorlie P, Steinberger J, Thom T, Wasserthiel-Smoller S and Hong Y (2007). Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* **115(5):**e69-171.

Samama MM (2002). Synthetic direct and indirect factor Xa inhibitors. *Thromb Res* 106(3):V267-273.

Samama MM and Gerotziafas GT (2003). Evaluation of the pharmacological properties and clinical results of the synthetic pentasaccharide (fondaparinux). *Thromb Res* **109(1):**1-11.

Schumacher W, Bostwick J, Stewart A, Steinbacher T, Pinto D and Wong P (2007). The direct factor Xa inhibitor apixaban produces broad antithrombotic activity with limited effects on bleeding time in rats. *Blood* **110(11):**A4005.

Simoons ML, Bobbink IW, Boland J, Gardien M, Klootwijk P, Lensing AW, Ruzyllo W, Umans VA, Vahanian A, Van De Werf F and Zeymer U (2004). A dose-finding study of fondaparinux in patients with non-ST-segment elevation acute coronary syndromes: the Pentasaccharide in Unstable Angina (PENTUA) Study. *J Am Coll Cardiol* **43(12):**2183-2190.

Strasberg SM, Harvey PR and Hofmann AF (1990). Bile sampling, processing and analysis in clinical studies. *Hepatology* **12(3 Pt 2):**176S-180S; discussion 180S-182S.

Walenga JM, Bara L, Petitou M, Samama M, Fareed J and Choay J (1988). The inhibition of the generation of thrombin and the antithrombotic effect of a pentasaccharide with sole anti-factor Xa activity. *Thromb Res* **51(1):**23-33.

Walenga JM, Jeske WP, Hoppensteadt D and Fareed J (2003). Factor Xa inhibitors: today and beyond. *Curr Opin Investig Drugs* **4(3):**272-281.

Wang L, Zhang D, Swaminathan A, Cheng PTW, Xue Y, Mosqueda-Garcia R, Everett DW, and Humphreys WG (2006) Glucuronidation as a major metabolic clearance pathway of [^{14}C]muraglitazar in humans: different metabolic profiles in subjects with or without bile collection. *Drug Metab Dispos* **34:** 427–439.

Wittkowsky AK (2004). Effective anticoagulation therapy: defining the gap between clinical studies and clinical practice. *Am J Manag Care* **10(10 Suppl):**S297-306; discussion S312-297.

Wong P, Watson C, Crain E, Luettgen J, Ogletree M, Wexler R, Lam P, Pinto D and Knabb R (2006). Effects of the factor Xa inhibitor apixaban on venous thrombosis and hemostasis in rabbits. *Blood* **108:**275a.

Wong PC, Crain EJ, Xin B, Wexler RR, Lam PYS, Pinto DJ, Luettgen JM and Knabb RM (2008) Apixaban, an oral, direct and highly selective factor Xa inhibitor: *in vitro*, antithrombotic and antihemostatic studies. *J Thromb Haemost* **6:**820-829.

Yusuf S, Mehta SR, Chrolavicius S, Afzal R, Pogue J, Granger CB, Budaj A, Peters RJ, Bassand JP, Wallentin L, Joyner C and Fox KA (2006). Comparison of fondaparinux and enoxaparin in acute coronary syndromes. *N Engl J Med* **354(14):**1464-1476.

Yusuf S, Mehta SR, Chrolavicius S, Afzal R, Pogue J, Granger CB, Budaj A, Peters RJ, Bassand JP, Wallentin L, Joyner C and Fox KA (2006). Effects of fondaparinux on mortality and reinfarction in patients with acute ST-segment elevation myocardial infarction: the OASIS-6 randomized trial. *JAMA* **295(13):**1519-1530.

Footnote:

This study was funded by Bristol-Myers Squibb and Pfizer.

Legends of Figures

Figure 1. Concentration versus time profiles for apixaban and total radioactivity from male healthy subjects with and without bile collection following 20 mg single oral dose of [^{14}C]apixaban

Figure 2. Radiochromatograms of pooled plasma samples from subjects of (A) Group 1 without bile collection and (B) Group 2 with bile collection after oral administration of [^{14}C]apixaban at the selected time points: 1, 6, 12, 24, and 48 h.

Figure 3. Radiochromatograms of pooled urine (0-240 h), bile (3-8 h), and fecal samples (0-240 h) from subjects of (A) Group 1 without bile collection and (B) Group 2 with bile collection after oral administration of [^{14}C]apixaban

Figure 4. Proposed metabolic pathways of apixaban in humans

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. Summary of pharmacokinetic parameters for total radioactivity and apixaban after a single 20 mg (108.8 μCi) oral solution dose of [^{14}C]apixaban in Group 1 (n=6) and Group 2 (n=4, with bile collection) male subjects

Group	**Pharmacokinetic Parameters**					
	C_{max} (ng/mL) (mean, %CV)	**AUC_{0-T}** (ng•h/mL) (mean, CV %)	**AUC_{∞}** (ng•h/mL) (mean, CV %)	**T_{max}** (h) Median (range)	**$t_{1/2}$** (h) Mean (SD)	**CL/F** (mL/h)
Total radioactivity						
All subjects (N=10)	509 (16)	5305 (24)	5872 (23)	1.00 (0.50–2.00)	8.2 (1.69)	3405
Group 1 (N=6)	513 (20)	5087 (26)	5664 (24)	1.00 (0.50–2.00)	8.2 (1.59)	3531
Group 2 (N=4)	504 (11)	5649 (22)	6198 (23)	1.25 (0.50–2.00)	8.2 (2.09)	3226
Apixaban						
All subjects (N=10)	469 (17)	4050 (25)	4101 (25)	1.00 (0.50–2.00)	12.7 (8.55)	4876
Group 1 (N=6)	480 (20)	4055 (30)	4100 (30)	1.00 (0.50–2.00)	12.2 (8.48)	4878
Group 2 (N=4)	453 (12)	4041 (20)	4103 (20)	1.25 (0.50–2.00)	13.5 (9.91)	4874

CV=coefficient of variation; C_{max}=maximum concentration; $AUC_{(0-T)}$=area under the concentration versus time curve to the last measurable concentration; $AUC_{(0-\infty)}$=area under the concentration versus time curve to infinity; T_{max}=time to maximum concentration; SD=standard deviation; $t_{½}$=half-life; CL = clearance; F = bioavailability; The mean was from geometric analysis

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Relative distribution (percent of sample) of radioactive metabolites in pooled plasma at selected time points after an oral dose of [^{14}C]apixaban in Group 1 (n=6) and Group 2 (n=4, with bile collection) male subjects

Metabolic ID	RT (min)	Group 1 (n=6)					Group 2 (n=4)				
		1 h	6 h	12 h	24 h	48 h	1 h	6 h	12 h	24 h	48 h
M1	24.4	1.64	15.8	28.4	36.0	41.5	1.56	14.9	27.5	33.9	46.8
M2	28.6	ND	ND	ND	ND	ND	ND	0.21	ND	ND	ND
M7	27.6	ND	0.75	ND	ND	ND	ND	0.67	0.30	ND	ND
M10	19.0	ND	0.34	ND	ND	ND	ND	0.76	0.67	ND	ND
Apixaban	48.9	98.4	83.2	71.6	64.0	58.5	98.4	83.5	71.5	61.0	53.2
Others		NA	NA	NA	NA	NA	NA	NA	NA	5.10	NA
Total		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

96-hour sample was not analyzed due to low radioactivity. RT=retention time; ND=not detected; NA=not applicable

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3. Estimated exposures to apixaban and O-demethyl apixaban sulfate (M1) following oral administration of [^{14}C]apixaban (20 mg) in Group 1 (n=6) and Group 2 (n=4, with bile collection) male subjects

Time (h)	Total radioactivity (dpm/mL)		[apixaban]* (μM)		[M1]* (μM)		AUC (apixaban)$_{T1-T2}$* (μM•h)		AUC (M1)$_{T1-T2}$* (μM•h)		AUC(M1)$_{0-48h}$ /AUC (apixaban)$_{0-48h}$	
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2
1	6120	7540	1.084	1.336	0.018	0.021	0.542	0.668	0.009	0.011		
6	5280	4780	0.791	0.718	0.150	0.128	4.687	5.135	0.421	0.373		
12	2750	2390	0.354	0.308	0.141	0.118	3.436	3.078	0.872	0.740		
24	935	885	0.108	0.097	0.061	0.054	2.773	2.429	1.207	1.034		
48	275	265	0.029	0.025	0.021	0.022	1.640	1.471	0.974	0.916		
0–48							13.078	12.780	3.483	3.073	0.2663	0.2405

*Concentration is estimated from the relative distribution of apixaban or M1 in the plasma, the total concentration of radioactivity and the specific activity (5.44 μCi/mg) of the administered drug; AUC_{T1-T2} is estimated from the concentration in plasma at limited time points (1, 6, 12, 24 and 48 hours) using the trapezoidal rule. dpm=disintegrations per minute; AUC=area under the plasma concentration versus time curve; G1=group 1; G2=group 2

DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Table 4. Relative distribution (percent of dose) of radioactive metabolites in pooled urine, bile and feces after an oral dose of [^{14}C]apixaban in Group 1 (n=6) and Group 2 (n=4, with bile collection) male subjects

Metabolic ID	RT (min)	Group 1		Group 2		
		Urine	Feces	Bile	Urine	Feces
M1	24.4	1.58	1.24	0.96	2.55	2.40
M2	28.6	ND	12.2	0.02	ND	5.09
M4	34.1	ND	0.37	ND	ND	ND
M7	27.6	1.46	3.70	0.32	1.85	2.76
M10	19.0	ND	0.09	0.24	0.46	0.26
M13	21.3	ND	3.07	0.06	ND	0.96
Apixaban	48.9	21.5	34.0	0.84	23.9	34.5
Others[a]	NA	NA	1.30	NA	NA	0.73
Total	NA	**24.5**	**56.0**	**2.44**	**28.8**	**46.7**

[a]Radioactivity distributed among many small peaks each representing ≤0.02%. RT=retention time; ND=not detected; NA=not applicable.

Fig 1

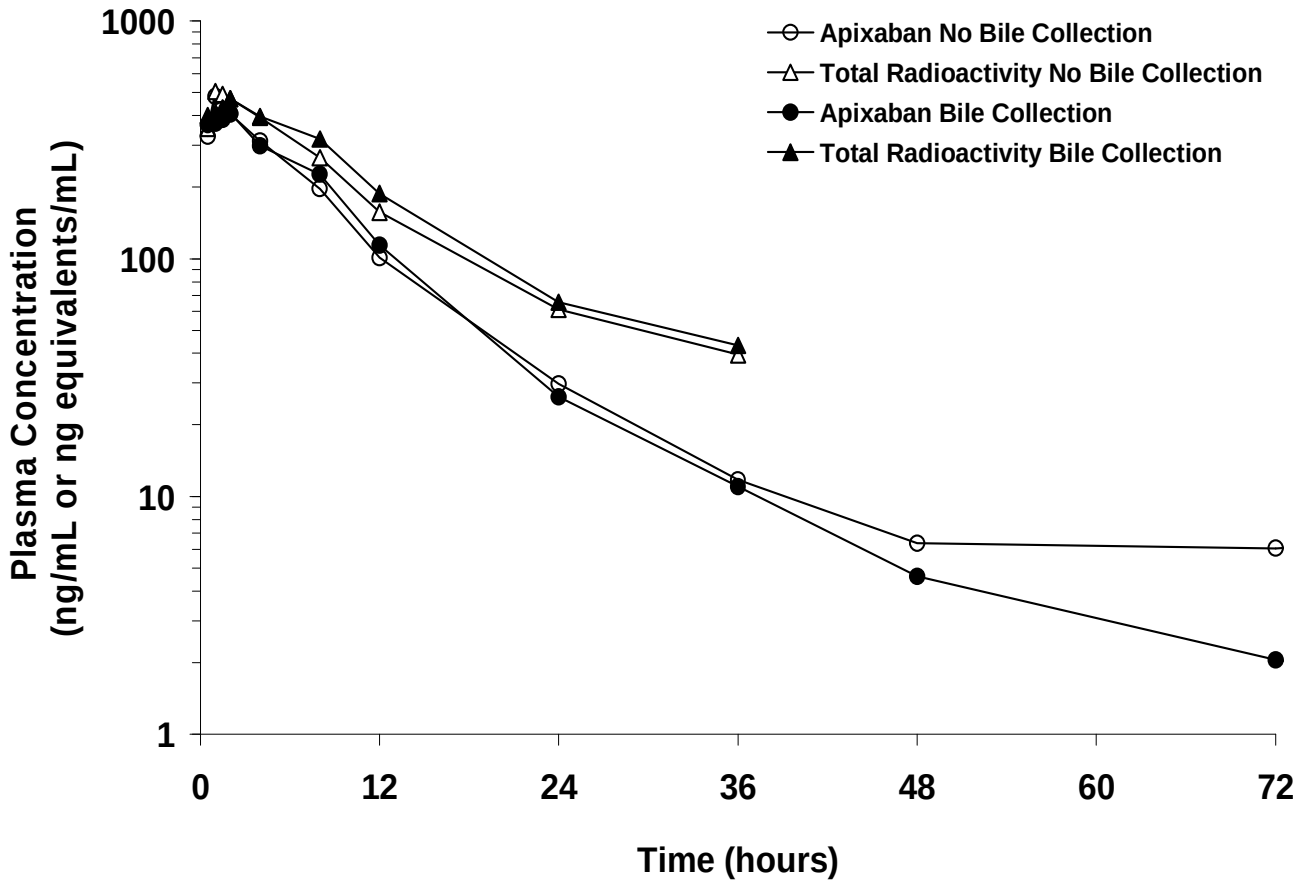


Fig 2A

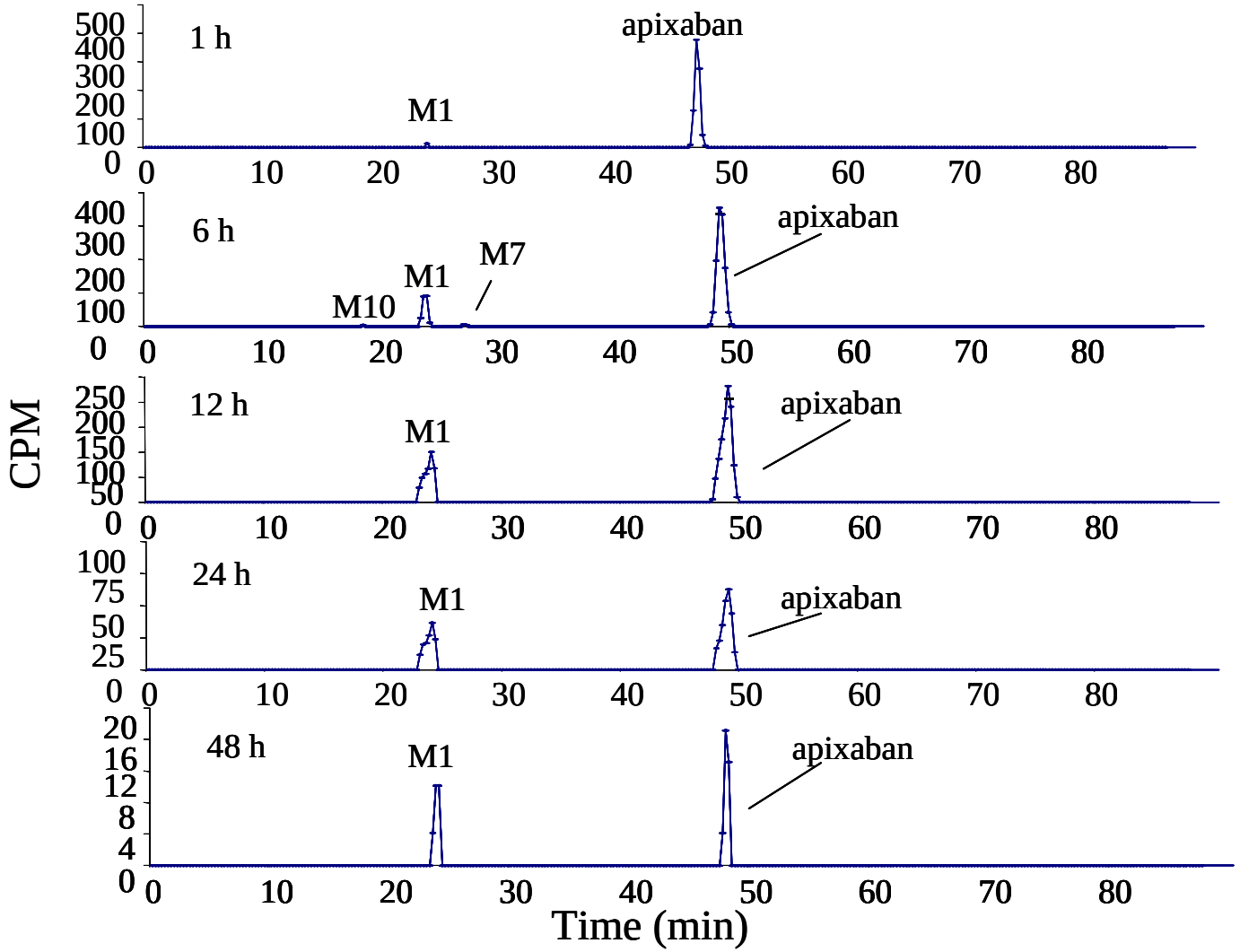


Fig 2B

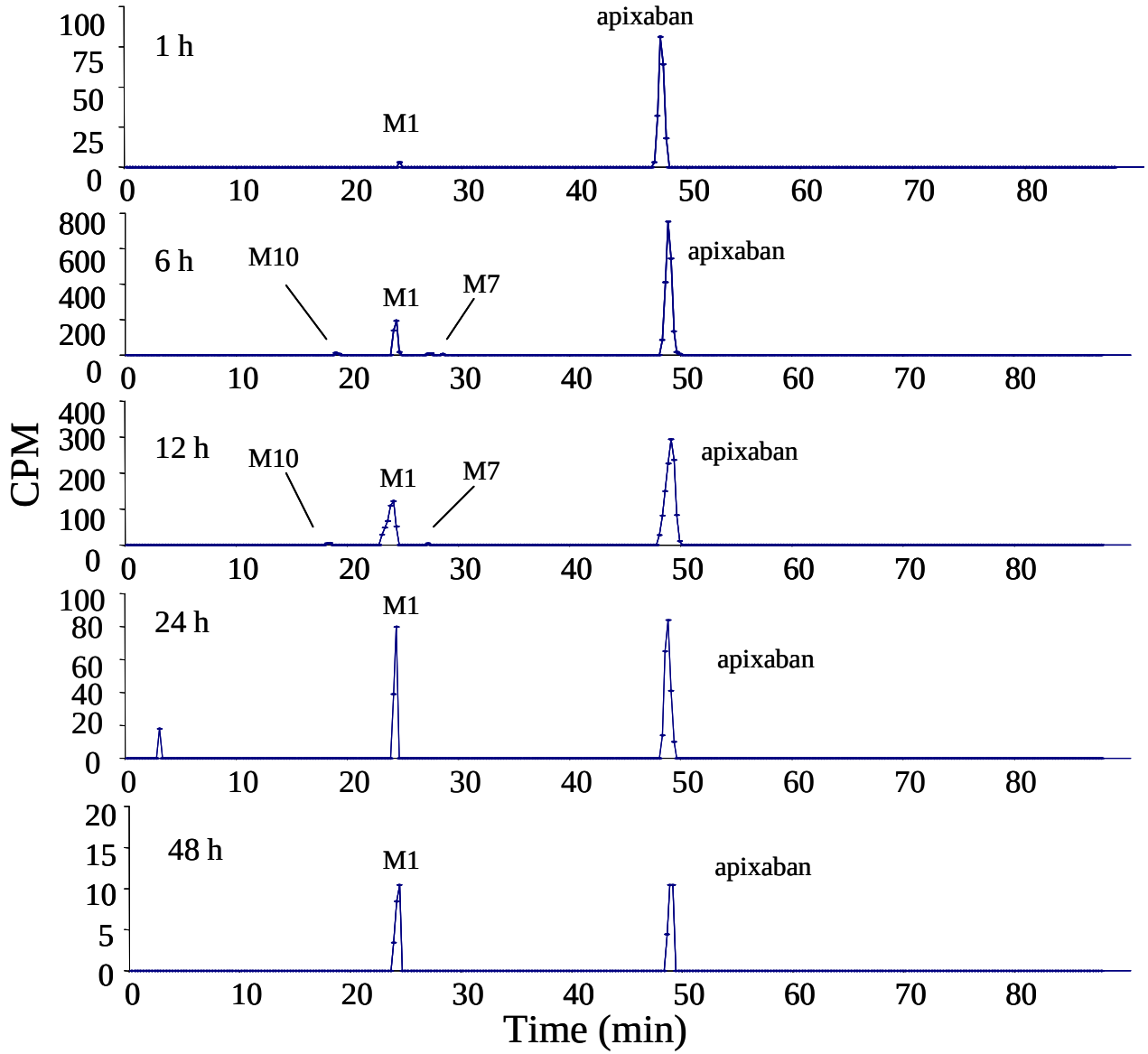


Fig 3A

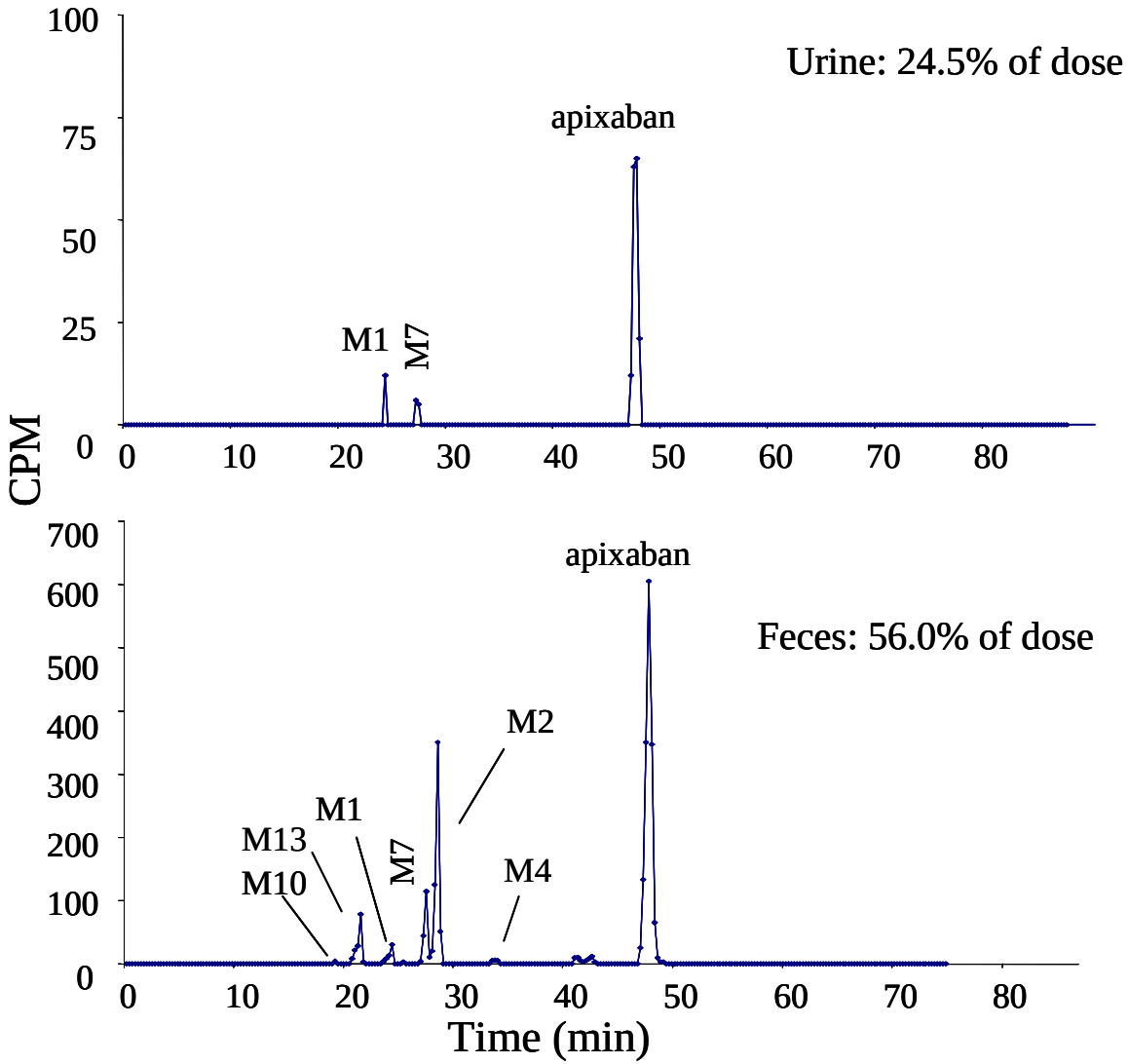
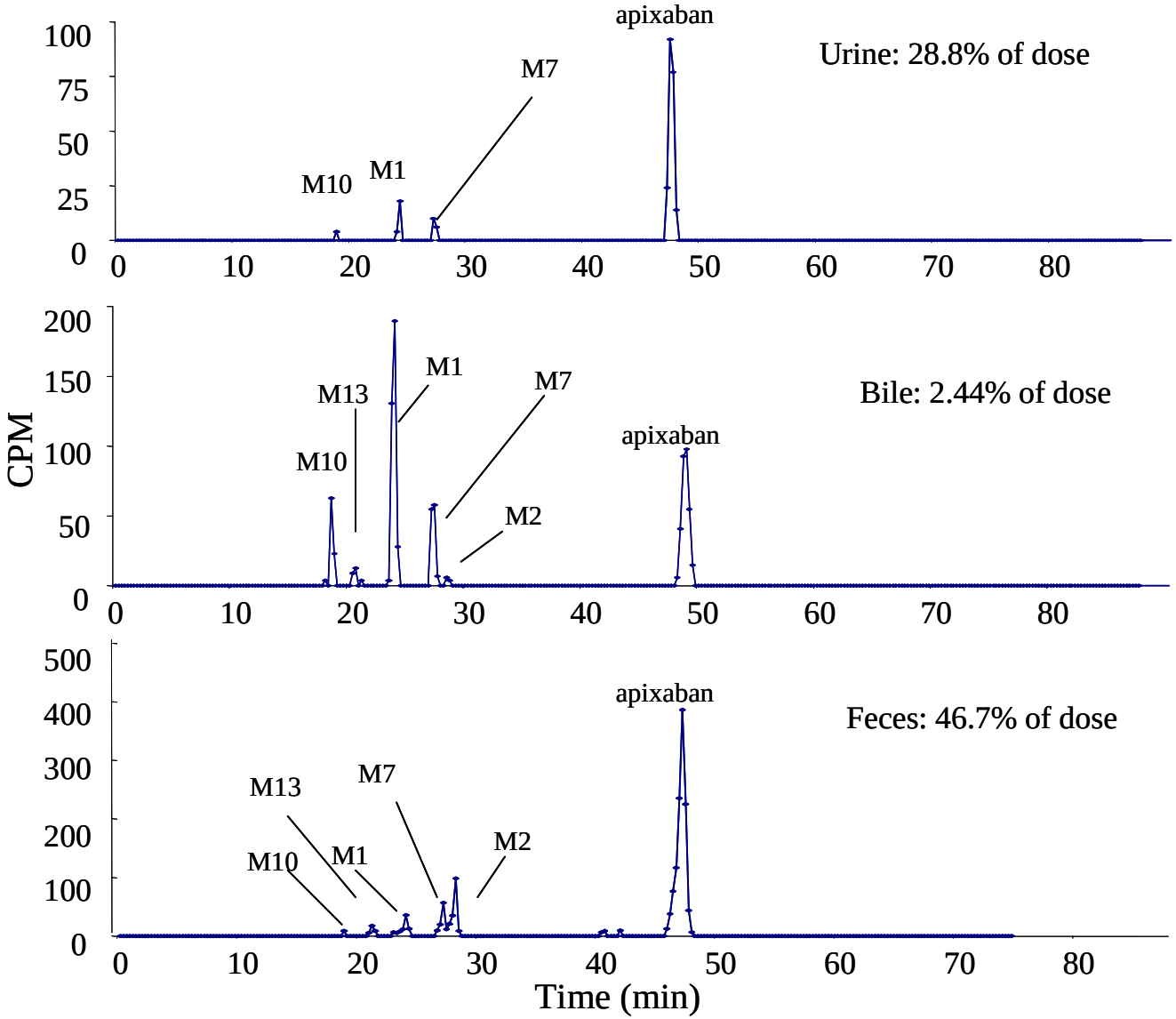


Fig 3B



DMD Fast Forward. Published on October 2, 2008 as DOI: 10.1124/dmd.108.023143
This article has not been copyedited and formatted. The final version may differ from this version.

Fig 4