Pharmacokinetics, Biotransformation, and Mass Balance of Edoxaban, a Selective, Direct Factor Xa Inhibitor, in Humans

Mohinder S. Bathala, Hiroshi Masumoto, Toshihiro Oguma, Ling He, Chris Lowrie, and Jeanne Mendell

Running title: PK and Mass Balance of Edoxaban

Corresponding author:
Jeanne Mendell
399 Thornall Street, Edison, NJ 08837 USA
Tel# 1-732-590-3432, fax # 1-732-906-5690
e-mail address: jmendell@dsi.com

Manuscript breakdown:
Pages: 25
Tables: 3
Figures: 5
References: 19
Abstract: 201 words
Introduction: 513 words
Discussion: 573 words

Nonstandard abbreviations: AE, adverse event; AUC, area under the curve; Cmax, maximum observed concentration; CYP, cytochrome P450; FXa, factor Xa; HPLC, high-performance liquid chromatography; LC-MS/MS, high-performance liquid chromatography/tandem mass spectrometry; MRM, multiple reaction monitoring; PK, pharmacokinetics.
Abstract

This study determined the mass balance and pharmacokinetics of edoxaban in humans after oral administration of \(^{[14}C\)-edoxaban. After oral administration of 60 mg (as active moiety) of \(^{[14}C\)-edoxaban to six healthy male subjects, serial blood/plasma, and urinary and fecal samples were collected for up to 168 h post-dose. All samples were analyzed for total radioactivity by liquid scintillation counting and for concentrations of edoxaban and four metabolites in plasma, urine, and fecal samples by either high-performance liquid chromatography/tandem mass spectrometry method using multiple reaction mode or a liquid chromatography radiometric method. The mean recovery of radioactivity was >97% of the administered radioactive dose, with 62.2% eliminated in feces and 35.4% in urine. Unchanged edoxaban accounted for the majority of radioactivity, with 49.1 and 23.8% of the dose as parent observed in feces and urine, respectively. Unchanged edoxaban was the most abundant species in plasma with a mean AUC\(_{0-\infty}\) of 1596 ng·h/ml. The next most abundant species was metabolite M4 with a mean AUC\(_{0-\infty}\) 147 ng·h/ml. The mass balance of edoxaban was well described with unchanged edoxaban as the most abundant component of total radioactivity. Edoxaban is eliminated through multiple pathways, but each accounts for only a small amount of overall elimination.
Introduction

Nonvalvular atrial fibrillation, which increases the risk of ischemic stroke and venous thromboembolism (Thomas and Lerman, 2011; Olesen et al., 2011), are major medical issues associated with significant morbidity and mortality (Cohen et al., 2007). Patients with these thrombotic disorders generally require extended thromboprophylaxis, which currently includes warfarin, despite its many limitations (Fuster, et al., 2011). Factor Xa (FXa) is a target for new anticoagulants and is located at the junction of the intrinsic and extrinsic pathways of coagulation. Free FXa is responsible for the generation of the first trace amounts of thrombin, and FXa within the prothrombinase complex (bound to the surface of activated platelets) induces the propagation phase of thrombin generation from prothrombin (Rai et al., 2001; Weitz, 2011).

Edoxaban (Fig. 1) is an oral, direct, selective FXa inhibitor (Furughori et al., 2008). The antithrombotic action of edoxaban has been demonstrated in vitro and in vivo in experimental models of arterial and venous thrombosis in different animal species (Furughori et al., 2008). In humans, the pharmacokinetics (PK) of edoxaban is characterized by rapid absorption (1–3 h) and an elimination half-life of about 8–10 h. Edoxaban demonstrates linear PK within the therapeutic dose range and food does not significantly alter its absorption (Ogata et al., 2010; Mendell et al., 2011). The safety of edoxaban has been demonstrated in patients with nonvalvular atrial fibrillation (Weitz et al., 2010) and efficacy and safety for prevention of venous thromboembolism in patients undergoing major orthopedic surgery (Fuji et al., 2010; Raskob et al., 2010). Edoxaban has recently been approved for thromboprophylaxis in patients undergoing orthopedic surgery in Japan. Edoxaban is also being studied in two phase III clinical trials. The Effective aNticoaGulation with factor xA next Generation in Atrial Fibrillation-Thrombolysis in
Myocardial Infarction study 48 (ENGAGE AF-TIMI 48; clinicaltrials.gov identifier NCT00783191) trial is evaluating the efficacy and safety of edoxaban for stroke prevention in patients with atrial fibrillation (Ruff et al., 2010). The Hokusai-Venous Thromboembolism (Hokusai-VTE; clinicaltrials.gov identifier NCT00986154) trial is the largest single, randomized, multinational phase III venous thromboembolism treatment and secondary prevention trial for patients with symptomatic deep vein thrombosis and/or pulmonary embolism (Daiichi Sankyo Inc., 2010).

After a single oral dose of 0.3-1 mg/kg to nonhuman primates, radiolabeled edoxaban is rapidly absorbed, with a bioavailability of 53.6 to 55.6% (Yoshigae et al., 2010). The amounts of total radioactivity found in urine and feces of monkeys were 42.0 and 51.0% of the dose, respectively, and the parent drug was the main component in plasma, urine, and feces (Yoshigae et al., 2010). The major metabolites of edoxaban found in the urine and/or feces in this experimental model were M8, M6, M2 (feces only), and M1 (urine and feces) (Yoshigae et al., 2010, data on file).

The objectives of this study were to assess the mass balance of edoxaban in humans, determine the PK of the parent drug and its known metabolites in plasma, urine, and feces, and to identify potential unknown metabolites of edoxaban in six healthy male subjects who received a single 60 mg oral dose of the radiolabeled drug.
Materials and Methods

Study Design. This was a single center, open-label, nonrandomized study with the administration of radiolabeled edoxaban to six healthy male subjects to determine the PK of the parent drug and known metabolites and to identify potential unknown metabolites. The study was conducted at Charles River Clinical Services (Edinburgh, UK). The study followed the guidelines of the World Medical Association Declaration of Helsinki in its revised edition, the guidelines for current Good Clinical Practice, the requirements of Directive 2001/20/EC2 and the corresponding UK statutory instrument, as well as the demands of national drug and data protection laws and other applicable regulatory requirements. All subjects provided written informed consent.

Subjects. Subjects were male (30–55 years of age, body mass index 19–29 kg/m²) and in good health, as determined by a medical history, physical examination, 12-lead electrocardiogram, and clinical laboratory evaluations in accordance with the inclusion criteria for this study. Subjects were excluded if they had received any prescribed systemic, herbal, or topical medication within 30 days of first-dose administration, or had any expectation of requiring use of any prescribed or topical medication while participating in the study. History of any clinically significant neurological, gastrointestinal, renal, hepatic, cardiovascular, psychological, pulmonary, metabolic, endocrine, connective tissue disease, autoimmune disease, hematological, or other major disorders, and positive for hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency virus, were also grounds for exclusion.
Dose Preparation and Administration. $[^{14}C]$-edoxaban was synthesized by BioDynamics Research Limited (now known as Quotient, Rushden, UK) and the radiolabel was located in a stable position on the carbonyl group of the tetrahydrothiazole-pyridinecarbonayl moiety of the edoxaban molecule (Fig. 1). The final dose of edoxaban tosylate was prepared by Charles River Laboratories (Edinburgh, UK) with addition of 29.1 mg (21.6 mg as free base) of $[^{14}C]$-edoxaban to 145.4 mg of edoxaban (108.0 mg as free base) in 50 ml of sterile water solution. The dose contained approximately 2.2 MBq (0.57 mSv) of radioactivity. This radiation exposure falls within the International Commission on Radiological Protection guidelines for category IIa studies (0.1 mSv to 1 mSv). The purity and specific activity were confirmed upon receipt and after preparation of test doses at hours 0, 24, and 48 post-preparation via high-performance liquid chromatography (HPLC) method to have a specific activity of 214.7 kBq/mg and radiochemical purity of 98.6%. After screening for eligibility, each subject was admitted on Day -1 and on Day 1, after a 4 h fast, received a single 60 mg dose of $[^{14}C]$-edoxaban as an oral solution followed by 240 ml of water used to rinse the dosing container at approximately 8:30. Subjects remained seated for 3 hours after dosing and then could resume normal activities, excluding strenuous activity, 4 hours after dosing. Each subject had a bowel movement each day with the exception of Subject 005 on Day 3. Subjects were discharged on Day 11, 240 h after dosing. Subjects received standard meals at approximately 9:00, 12:30 and 17:30 of each day during the confinement in clinic with the exception of Day 1 where the first meal was lunch. Subjects were restricted from caffeinated beverages, grapefruit juice, alcohol, and concomitant medications.

Collection and Preparation of Blood/Plasma, Urine, and Fecal Samples. Serial blood samples (12 ml) were collected at 0 (predose), 0.5, 1, 1.5, 2, 4, 8, 12, 15, 24, 36, 48, 72, 96, 120,
144, and 168 h post-dose. Each blood sample was gently mixed and approximately 10 mL of the 12 ml were placed into a lithium heparin tube. After centrifugation at 1500 g for 10 min at approximately 4°C, the separated plasma was transferred into two polypropylene tubes: an aliquot for total radioactivity and an aliquot for measurement of edoxaban and metabolites. The remaining 2 ml of whole blood from the 12 ml sample was placed into a 2 ml lithium heparin tube and stored at approximately 2° to 8°C until analyzed for total radioactivity. Additional 25 ml blood samples were collected at 0 (predose), 1, 2, 4, 8, 12, 24, 48, 72, and 96 h post-dose for metabolite profiling. Urine samples were collected and pooled at 0 (predose), 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–72, 72–96, 96–120, 120–144, and 144–168 h post-dose. Four 20 ml samples were aliquoted from pooled and weighed total volume at each timepoint. Fecal samples were collected and pooled at 0 (predose), 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 h post-dose. Fecal homogenates (2 × ~50 g) were obtained from total pooled and weighed samples collected at each timepoint.

**Radiometric Analysis of Edoxaban and Metabolites.** All samples were processed appropriately and radioactive contents of extracts were determined using duplicate aliquots. The aliquots were admixed with scintillation fluid, and subjected to liquid scintillation counting for 5 min together with representative blank samples, using a liquid scintillation analyzer with automatic quench correction by an external standard method. The lower limit of detection was 30 dpm above background. Recovery of radioactivity in urine and feces collected from each subject during the course of the trial was calculated as percent of dose administered, and cumulative recovery of radioactivity was determined. Radioactivity concentrations in whole
blood and plasma collected from each subject during the course of the trial were calculated in μg equiv/ml of edoxaban.

Radiochromatographic analyses of processed plasma, whole blood, urine, and fecal samples were performed using reverse-phase HPLC by Charles River Laboratories (Trantent, UK) to quantitate concentrations of edoxaban and known metabolites in plasma, urine, and feces, with further analysis by Radiation Safety and Control Group, Daiichi Sankyo (via Japan Radioisotope Association, Tokyo, Japan). Standards of edoxaban and the known metabolites (M1, M4, M6, and M8) were analyzed to establish retention times and multiple reaction monitoring (MRM) transitions for each analyte to identify peaks in the MRM chromatogram and radio-HPLC chromatograms. Reference metabolite standards were synthesized with structural confirmation by nuclear magnetic resonance and high resolution mass spectrometric analyses and provided by the study sponsor (Daiichi Sankyo, Tokyo, Japan). The lower limit of quantitation for the radio-HPLC method was 14 ng equiv/g. The metabolites M2, M3, and M5 were identified based on MRM chromatograms and compared with standards synthesized for these metabolites. The identification of M7 is putative and was speculated in the monkey metabolism study (data on file). M7 is thought to be a precursor to M8 and has not yet been synthesized. These metabolites were identified to complete a thorough characterization of possible metabolites and the postulated metabolic pathway, but were not quantitated since only trace amounts were observed.

**HPLC/Tandem Mass Spectrometry Analysis of Edoxaban and Metabolite Concentrations.**

The validated bioanalytical method by high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) for the measurement of edoxaban in plasma and urine was developed
DMD #46888

by BioDynamics. The analytes were extracted from the biological matrix using liquid-liquid extraction. Extracts were measured by LC-MS/MS equipped with a turbo ion spray interface. The mass spectrometer was operated using MRM in the positive ion detection mode. The lower limits of quantitation for edoxaban concentrations in plasma and urine were 1 ng/ml and 20 ng/ml, respectively.

The metabolite (M1, M4, M6, and M8) concentrations in plasma were measured by a fit-for-purpose LC-MS/MS assay developed by Biodynamics. The metabolites were extracted from plasma using solid-phase extraction and then the concentrations were measured using an LC-MS/MS equipped with a turbo ion spray interface. The two stable isotopes used as internal standards were deuterated M1 and M6. The mass spectrometer was operated using MRM in the positive ion detection mode. The lower limit of quantitation for the metabolites in plasma was 50 pg/ml.

Safety. Safety was evaluated by recording adverse events (AEs), clinical laboratory assessments, recording of vital signs, physical examinations, and electrocardiograms.

Data Analysis. The following PK parameters were derived from concentration data for, where possible, total radioactivity (plasma and whole blood), edoxaban (plasma), and its metabolites (plasma): area under the curve (AUC_{0-\tau}, AUC_{0-\infty}), maximum observed concentration (C_{\text{max}}), time to reach maximum observed concentration (T_{\text{max}}), terminal elimination half-life (t_{1/2}), total apparent clearance (CL/f), and apparent volume of distribution (Vd/f). Total amount excreted in urine, percent dose excreted in urine, and renal clearance was estimated from urine edoxaban concentrations. The noncompartmental PK analysis for edoxaban and its metabolites was
performed by Biodynamics using WinNonlin, version 4.1 (Pharsight Corp., Mountain View, CA). The data were summarized statistically. The ratio of plasma AUC of edoxaban to that of total radioactivity was calculated and summarized. The excretion percentages of the various analytes, calculated from total radioactivity, were also summarized by Charles River Laboratories.
Results

Subjects. Eleven subjects were screened for the trial; six were enrolled and all completed the study. The mean (standard deviation [SD]) age of the subjects was 38.5 (4.9) years. The mean (SD) body weight at screening was 85.0 (5.6) kg and body mass index ranged from 25.0 to 28.8 kg/m². All six subjects were Caucasian males.

Absorption of Radioactivity. The maximum concentration of total radioactivity was observed at 0.5 h post-dose in five of the six subjects and at 1.0 h post-dose in the remaining subject in both plasma and whole blood, indicating rapid absorption of [¹⁴C]-edoxaban. The PK parameters for total radioactivity are summarized in Table 1. The cumulative urinary, fecal, and total radioactivity versus time are shown in Fig. 2. The mean (range) total radioactivity recovered in urine and feces was 97.6% (92.5–102.1%) at 168 h post-dose (Table 2). The mean cumulative radioactivity excreted in feces was 62.2% (55.2–73.0%), accounting for the majority of total radioactivity. The mean cumulative urinary radioactivity was 35.4% (27.4–46.9%) of the administered dose at 168 h post-dose. By 72 h post-dose, 85% of the total radioactivity had been excreted either in feces or urine.

The ratios of plasma edoxaban AUC to total radioactivity were close to unity, suggesting that most of the radiolabeled moiety persisted as parent drug. In whole blood, approximately 40% of the total radioactivity was associated with the cellular component.
Fecal and Urinary Excretion of Edoxaban and Metabolites. Edoxaban was also the predominant species excreted in urine (Table 2), accounting for 23.8% of the administered radioactivity. Urinary metabolites included M1, M4 (co-eluted with another analyte), M6, and a number of minor identified or unidentified radiolabeled components. Each urine metabolite represented <2% of total radioactivity. The most abundant metabolite detected in urine was M1, which represented a mean of 1.85% of total radioactivity collected over the 168 h study period. Therefore, a quantitative method was developed to measure urinary edoxaban concentrations.

The measured urine edoxaban concentrations versus time indicate that the majority of edoxaban is eliminated renally within 24 h. The mean (SD) cumulative amount of edoxaban detected in urine was 22.6 (±5.2) mg with an estimated median renal clearance of 12.7 l/h.

Edoxaban was also the predominant excreted component in feces (Table 2), accounting for 49.1% of the administered dose. The other identified peaks were M4, M6, and M8, with other more minor peaks observed. Each fecal metabolite represented <2% of total radioactivity. Due to these low radioactivity measures, the ratios of the metabolites to parent concentrations could not be determined.

Plasma Pharmacokinetics of Edoxaban and Its Metabolites. Plasma concentrations of edoxaban and its metabolites versus time course are shown in Fig. 3. Plasma edoxaban is the predominant species and shows a rapid peak with biexponential decline (Fig. 3A). The plasma metabolite concentrations follow the time course of the parent drug, albeit at much lower plasma concentrations. The most abundant plasma metabolite was M4 followed by M1 and M6, all with
peak concentrations < 25 ng/mL and rapid elimination phases as demonstrated (Fig. 3B). The plasma PK parameters for both parent drug and metabolites are summarized in Table 3. The peak plasma edoxaban concentration was 332 ng/ml observed at 0.5 h post-dose and $\text{AUC}_{(0-\infty)}$ was observed as 1596 ng·h/ml. The most abundant metabolite was M4 with $C_{\text{max}}$ and $\text{AUC}_{(0-\infty)}$ values of 22.2 ng/ml and 147.0 ng·h/ml, respectively. The M4 metabolite-to-parent ratio for $\text{AUC}_{(0-\infty)}$ was <10%, including the adjustment for molecular mass (ie, M4: 520.99 and edoxaban [anhydrous free base]: 548.06). The $t_{1/2}$ was similar to that of the parent. The metabolite M1 was the next most abundant metabolite, with $C_{\text{max}}$ and $\text{AUC}_{(0-\infty)}$ values of 14.9 ng/ml and 94.7 ng·h/ml, respectively. Similar to M4, M1 demonstrated a median $T_{\text{max}}$ value of 1.8 h. The metabolites M6 and M8 demonstrated the lowest abundance of the five measured analytes. Similar terminal elimination half-lives of approximately 9 h were observed for both M6 and M8.

The metabolites M2, M3, and M5 were identified based on MRM chromatograms. Standards were synthesized for these metabolites and MRM chromatogram results were confirmed. However, these metabolites were only observed in trace amounts and were not quantified. The representative chromatograms for each metabolite along with corresponding molecular structure, retention times, nominal mass and transitions are presented in Fig. 4.

**Safety.** There were no serious AEs and no subject was withdrawn from the study as a result of an AE. Three subjects (50.0%) experienced a total of nine AEs (one subject had 1 AE, one subject had 3 AEs, and one subject had 5 AEs). Six of these AEs were considered by the principal investigator to be possibly related to edoxaban. The most common AE was abdominal pain (two subjects). There was one bleeding-related AE (epistaxis), which was considered to be possibly
related to edoxaban. There were no clinically important changes in clinical laboratory values, vital signs, physical examinations, or electrocardiograms.
Discussion

This study of $^{14}$C-edoxaban had approximately 97% recovery of the administered radioactivity, indicating that the mass balance of edoxaban was well characterized. The most abundant species was the unchanged parent, edoxaban, with approximately 73% of total radioactivity detected in urine and feces. As expected, edoxaban was also the predominant component in plasma. The majority of the orally administered radioactive dose was detected in the feces, representing unabsorbed drug and biliary excretion of both the parent and metabolites. Approximately 35% of radioactive dose was eliminated in the urine, indicating that renal excretion is also an important route of elimination. Most of the urinary elimination of edoxaban occurred within the first 24 h, with 70% of the total radioactive amount excreted in urine within 8 h. Renal clearance of edoxaban was calculated as median of 12.7 l/h, indicating active secretion of the parent compound. Fecal elimination accounted for 62.2% of the total radioactivity. A recently completed absolute bioavailability study demonstrated that oral absorption of edoxaban is approximately 61.8% (Matushima et al., 2011). Therefore the unchanged edoxaban detected in the feces was a result of both unabsorbed drug and hepatobiliary excretion of systemically absorbed drug.

Four metabolites were identified and associated radioactivity was quantified in plasma, urine, and feces with measurement of metabolite concentrations in plasma. The total exposure (AUC values) for each of the four metabolites were <10% of parent exposure, defining them as minor metabolites (Center for Drug Evaluation and Research, 2008). The most abundant metabolite, M4, had metabolite:parent ratios of approximately 9.0%. The amount of total metabolites detected in urine and feces was approximately 5 and 4%, respectively, indicating further
metabolism of these metabolites and/or small volumes of distribution corresponding to higher plasma concentrations relative to those in urine and feces. Overall, metabolism appears to play a modest role in the elimination of edoxaban.

In vitro metabolism studies with human liver microsomes and recombinant cytochrome P450 (CYP) enzymes indicate that M1 and M4 are formed through hydrolysis while M6 and M8 through CYP3A (Masumoto et al., 2010). The postulated metabolic pathway is presented in Fig. 5. Both M6 and M8 were minor metabolites based on plasma exposure, approximately <4% of parent exposure. Therefore, CYP enzymes appear to have an insignificant role in the metabolism of edoxaban. The metabolites M1 (hydrolysis elimination of 5-chloropyridin-2-yl-oxamoyl group) and M4 (hydrolysis elimination of N, N-dimethylcarbamoyl group) were produced both in the presence and absence of NADPH, indicating that these two metabolites are formed independently of NADPH in human liver microsomes and formed through hydrolysis. Other metabolites, M2, M3, and M5 were identified and observed in trace amounts. M1 and M4 were the most abundant metabolites, indicating that hydrolysis is the predominant route of metabolism for edoxaban.

Slightly greater total systemic exposure (based on AUC) to total radioactivity was observed in plasma compared with whole blood, and an estimated 40% of the radioactivity was associated with the cellular component of whole blood. The mean plasma exposure and terminal elimination half-life values were similar to those reported for unlabeled drug in prior studies (Mendell et al., 2011; Ogata et al., 2010).
In summary, the mass balance of edoxaban was well characterized in this study with nearly complete recovery of the total radioactivity. Unchanged edoxaban was the most abundant species in plasma, urine, and feces, with modest metabolism (<25% total dose) that occurs primarily through hydrolysis. Edoxaban is eliminated through multiple elimination pathways including renal excretion, biliary excretion, and metabolism.
Acknowledgments

The authors would like to acknowledge Evince Communications for assistance in editing the manuscript, ensuring version control, and preparing and formatting the bibliography. Editorial assistance was supported by Daiichi Sankyo.

Authorship Contributions

Participated in research design: (Bathala, Masumoto)
Conducted experiments: (Lowrie)
Contributed new reagents or analytic tools: (Masumoto, Oguma)
Performed data analysis: (He, Lowrie)
Wrote or contributed to the writing of the manuscript: (Bathala, Masumoto, Oguma, He, Lowrie, Mendell)
References


Footnotes

Dr Bathala has since retired from Daiichi Sankyo.

Meeting abstracts where the work was previously presented:


Requests for reprints to:
Jeanne Mendell
399 Thornall Street, Edison, NJ 08837 USA
Tel# 1-732-590-3432, fax# 1-732-906-5690
e-mail address: jmendell@dsi.com
Figure Legends

FIG. 1. Molecular structure of edoxaban and position of $^{14}$C-label (*).

Total activity: 71.3 MBq
Specific activity: 214.7 kBq/mg >98% radiochemical purity
Mass: 622.2 mg >98% chemical purity

FIG. 2. Cumulative excretion of total radioactivity in urine, feces, and combined urine and feces following a single oral dose of 60 mg $^{14}$C-edoxaban.

FIG. 3. Plasma concentrations of edoxaban and metabolites (A) and metabolites only (B) following a single oral dose of 60 mg $^{14}$C-edoxaban.

FIG. 4. Representative chromatograms for each metabolite, corresponding molecular structure, retention times (min), nominal mass (ion m/z) and transition established in multiple-stage mass spectrometry.

FIG. 5. Postulated metabolic pathway for edoxaban. HF, human feces; HP, human plasma; HU, human urine; UGT, uridine 5'-diphospho-glucuronosyltransferase; CES1, carboxylesterase 1; CYP3A4/5, cytochrome P450 isoenzyme 3A4/5.
## Tables

### TABLE 1

**Pharmacokinetic parameters for total radioactivity in whole blood and plasma**

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg equiv/ml)</th>
<th>AUC&lt;sub&gt;0-τ&lt;/sub&gt; (µg equiv·h/ml)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µg equiv·h/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometric Mean</td>
<td>(CV%)</td>
<td>Geometric Mean</td>
<td>Geometric Mean</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>(µg equiv/ml)</td>
<td>(CV%)</td>
<td>(µg equiv·h/ml)</td>
<td>(µg equiv·h/ml)</td>
<td>(range)</td>
<td>(SD)</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td>0.349 (35.6)</td>
<td>1.683 (23.2)</td>
<td>1.947 (20.5)</td>
<td>0.50</td>
<td>4.40</td>
</tr>
<tr>
<td><strong>Whole Blood</strong></td>
<td>0.352 (36.8)</td>
<td>1.269 (50.5)</td>
<td>1.673 (46.1)</td>
<td>0.50</td>
<td>3.81</td>
</tr>
</tbody>
</table>

AUC, area under the curve; C<sub>max</sub>, maximum observed concentration; CV, coefficient of variation; t<sub>1/2</sub>, terminal elimination half-life; T<sub>max</sub>, time to reach maximum observed concentration; SD, standard deviation.
### TABLE 2

**Mean cumulative percentage of total radioactivity detected in feces and urine by analyte (n = 6)**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean % Dose Detected in Feces</th>
<th>Mean % Dose Detected in Urine</th>
<th>Total Recovery (% Dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Dose</td>
<td>62.2</td>
<td>35.4</td>
<td>97.6</td>
</tr>
<tr>
<td>Edoxaban</td>
<td>49.1</td>
<td>23.8</td>
<td>72.8</td>
</tr>
<tr>
<td>M6</td>
<td>1.66</td>
<td>0.45</td>
<td>2.11</td>
</tr>
<tr>
<td>M8</td>
<td>0.34</td>
<td>BLQ</td>
<td>0.34</td>
</tr>
<tr>
<td>M1</td>
<td>BLQ</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>M4</td>
<td>1.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.49</td>
</tr>
<tr>
<td>Other minor unknown peaks</td>
<td>0.05</td>
<td>1.37</td>
<td>ND</td>
</tr>
</tbody>
</table>

BLQ, below limit of quantitation; ND, not determined; % Dose, dose excreted in urine.

<sup>a</sup> Co-eluted with another analyte in radiochromatogram.
TABLE 3

Mean (SD) plasma pharmacokinetic parameters for edoxaban and its metabolites

<table>
<thead>
<tr>
<th>Analyte</th>
<th>N</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (h)$^a$</th>
<th>$\text{AUC}_{0-\tau}$ (ng·h/mL)</th>
<th>$\text{AUC}_{0-\infty}$ (ng·h/mL)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edoxaban</td>
<td>6</td>
<td>332 (108)</td>
<td>0.5 (0.5, 0.5)</td>
<td>1584 (305)</td>
<td>1596 (305)</td>
<td>7.4 (1.7)</td>
</tr>
<tr>
<td>M6</td>
<td>6</td>
<td>6.99 (2.97)</td>
<td>1.0 (0.5, 1.0)</td>
<td>55.1 (16.7)</td>
<td>56.2 (16.7)</td>
<td>9.0 (1.9)</td>
</tr>
<tr>
<td>M8</td>
<td>6</td>
<td>0.63 (0.349)</td>
<td>1.8 (0.5, 2.0)</td>
<td>3.50 (1.74)</td>
<td>4.41 (1.80)</td>
<td>9.0 (4.6)</td>
</tr>
<tr>
<td>M1</td>
<td>6</td>
<td>14.9 (2.22)</td>
<td>1.8 (0.5, 2.0)</td>
<td>94.7 (16.7)</td>
<td>95.8 (16.8)</td>
<td>6.9 (1.7)</td>
</tr>
<tr>
<td>M4</td>
<td>6</td>
<td>22.2 (9.70)</td>
<td>1.8 (1.5, 2.0)</td>
<td>145.5 (46.9)</td>
<td>147.0 (46.8)</td>
<td>8.2 (1.1)</td>
</tr>
</tbody>
</table>

$^a$ Median (min, max).

SD, standard deviation; AUC, area under the curve; $C_{\text{max}}$, maximum observed concentration; $t_{1/2}$, terminal elimination half-life; $T_{\text{max}}$, time to reach maximum observed concentration.
Figure 1

Edoxaban
Figure 2

Graph showing the % Administered dose over time (h) for Matrix, Urine, Feces, and Total Recovery.
Figure 4

<table>
<thead>
<tr>
<th>Standard</th>
<th>Ion Retention Time (min)</th>
<th>Ion m/z [M+H]+</th>
<th>Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>25.41</td>
<td>438 ([M-Li+2H]+)</td>
<td>438→376</td>
</tr>
<tr>
<td>M2</td>
<td>34.09</td>
<td>366</td>
<td>366→152</td>
</tr>
<tr>
<td>M3</td>
<td>38.00/38.90</td>
<td>724</td>
<td>724→548</td>
</tr>
<tr>
<td>M4</td>
<td>40.84</td>
<td>521</td>
<td>521→339</td>
</tr>
<tr>
<td>M5</td>
<td>41.45/42.00</td>
<td>564</td>
<td>564→382</td>
</tr>
<tr>
<td>M6</td>
<td>46.52</td>
<td>534</td>
<td>534→352</td>
</tr>
<tr>
<td>M8</td>
<td>49.97</td>
<td>534</td>
<td>534→352</td>
</tr>
<tr>
<td>edoxaban</td>
<td>54.08</td>
<td>548</td>
<td>548→366</td>
</tr>
</tbody>
</table>
Figure 5

The diagram illustrates the metabolic pathways of Edoxaban, a direct oral anticoagulant. The following metabolites and enzymes are involved:

- **M2** (HP, HU): Metabolite produced by hydrolysis of Edoxaban.
- **M1** (HP, HU): Metabolite produced by CYP3A4/5 metabolism of M2.
- **M3** (HP, HU): Metabolite produced by hydrolysis of M2 and conjugated by UGT.
- **M4** (HP, HU, HF): Metabolite produced by CES1 metabolism of M3.
- **M5** (HP, HU, HF): Metabolite produced by CYP3A4/5 metabolism of M2.

The pathways highlight the importance of enzymes such as CYP3A4/5 and CES1 in the metabolism of Edoxaban, leading to the formation of multiple active and inactive metabolites.