

**Pharmacokinetics and ADME characterization of intravenous and oral [<sup>14</sup>C]-linerixibat  
in healthy male volunteers**

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## Running Title Page

**Running Title:** PK and ADME of IV and oral linerixibat in healthy volunteers (45/60 characters)

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Target journal: [DMD](#)

Number of text pages: 30

Number of Figures: 6

Number of Tables: 3

References: 31

Abstract: 249/250

Significance Statement: 57/80

Introduction: 550/750

Discussion: 1203/1500

### Abbreviations:

ADME, absorption, distribution, metabolism, and excretion; AMS, accelerator mass spectrometry; AUC, area under the plasma concentration-time curve; BMI, body mass index; CI, confidence interval;  $Cl_{h,iv,plasma}$ , hepatic plasma clearance;  $Cl_{iv}$ , total plasma clearance;

$Cl_{\text{renal,iv}}$ ; renal clearance;  $Cl_{\text{h,iv,blood}}$ , hepatic blood clearance;  $C_{\text{max}}$ , maximum observed plasma concentration; CVb, between participant variability; CYP, cytochrome P450; DDI, drug-drug interaction; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid;  $E_{\text{h}}$ , hepatic extraction ratio; F, absolute oral bioavailability;  $f_{\text{a}}$ , fraction of linerixibat absorbed;  $f_{\text{g}}$ , fraction escaping first-pass gut metabolism;  $F_{\text{h}}$ , first pass liver extraction; GI, gastrointestinal; IBAT, ileal bile acid transporter;  $IC_{50}$ , half maximal inhibitory concentration; ICRP, International Commission on Radiological Protection; IV, intravenous; IVIVE, *in vitro*-to-*in vivo* extrapolation; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLQ, lower limit of quantification; LSC, liquid scintillation counting; PBC, primary biliary cholangitis; PK, pharmacokinetics; QC, quality control;  $Q_{\text{h}}$ , hepatic blood flow; SD, standard deviation;  $t_{1/2}$ , half-life;  $V_{\text{ss}}$ , volume of distribution;  $V_{\text{z}}$ , terminal volume

**Abstract (249/250 words)**

Linerixibat, an oral small molecule ileal bile acid transporter inhibitor under development for cholestatic pruritus in primary biliary cholangitis, was designed for minimal intestinal absorption (site of pharmacological action). This study characterized the pharmacokinetics, absorption, distribution, metabolism, and excretion of [<sup>14</sup>C]-linerixibat in humans following an intravenous microtracer, concomitant with unlabeled oral tablets, and [<sup>14</sup>C]-linerixibat oral solution. Linerixibat exhibited absorption-limited flip-flop kinetics: longer oral versus intravenous half-life (6–7 h vs 0.8 h). The short intravenous half-life was consistent with high systemic clearance (61.9 L/h) and low volume of distribution (16.3 L). *In vitro* studies predicted rapid hepatic clearance via cytochrome P450 (CYP) 3A4 metabolism, which predicted human hepatic clearance within 1.5-fold. However, linerixibat was minimally metabolized in humans after intravenous administration: ~80% elimination via biliary/fecal excretion (>90–97% as unchanged parent) and ~20% renal elimination by glomerular filtration (>97% as unchanged parent). Absolute oral bioavailability of linerixibat was exceedingly low (0.05%), primarily due to a very low fraction absorbed (0.167%;  $f_{\text{gut}} \sim 100\%$ ), with high hepatic extraction ratio (77.0%) acting as a secondary barrier to systemic exposure. Oral linerixibat was almost entirely excreted (>99% recovered radioactivity) in feces as unchanged and unabsorbed linerixibat. Consistent with the low oral fraction absorbed and ~20% renal recovery of intravenous [<sup>14</sup>C]-linerixibat, urinary elimination of orally administered radioactivity was negligible (<0.04% of dose). Linerixibat unequivocally exhibited minimal gastrointestinal absorption and oral systemic exposure. Linerixibat represents a unique example of high CYP3A4 clearance *in vitro*, but nearly complete excretion as unchanged parent drug via the biliary/fecal route.

**Significance Statement (57/80 words)**

This study conclusively established minimal absorption and systemic exposure to orally administered linerixibat in humans. The small amount of linerixibat absorbed was eliminated efficiently as unchanged parent drug via the biliary/fecal route. The hepatic clearance mechanism was mis-predicted to be mediated via cytochrome P450 3A4 metabolism *in vitro* rather than biliary excretion of unchanged linerixibat *in vivo*.

## Introduction

Primary biliary cholangitis (PBC) is a rare, chronic autoimmune liver disease caused by immune-mediated destruction of the intrahepatic bile ducts. This results in impaired bile acid flow and retention in the liver, leading to hepatic scarring, fibrosis and ultimately cirrhosis and liver failure (Boonstra et al., 2012; Gao et al., 2015). Cholestatic pruritus is a major symptom of PBC that significantly affects a patient's quality of life (Gotthardt et al., 2014; Hegade et al., 2015a; Jin and Khan, 2016; Hegade et al., 2019a; Lindor et al., 2019). The currently recommended treatments for pruritus can lead to symptomatic improvement in a subset of patients; however, a sizable population remain refractory and require the utilization of invasive experimental treatments such as nasobiliary drainage or even liver transplantation (Trivella and Levy, 2021). As such, an effective anti-pruritic drug therapy is an unmet clinical need in PBC (Trivedi et al., 2017).

Several putative pruritogens have been proposed in the treatment of PBC, including circulating bile acids (Hegade et al., 2015b), suggesting that the ileal bile acid transporter (IBAT), a solute carrier family transporter that mediates bile acid uptake from the gut lumen into enterocytes, may be a potential target for pruritus therapy (Hegade et al., 2015b; Al-Dury and Marschall, 2018). Inhibition of IBAT decreases bile acid reabsorption from the gastrointestinal (GI) tract, leading to decreased bile acid levels in the circulation and increased fecal bile acid excretion (Hegade et al., 2017; Hegade et al., 2019b). Alongside pruritus, IBAT inhibition could benefit diabetic dyslipidemia by decreasing bile acid reabsorption and forcing the liver to increase *de novo* bile acid synthesis from cholesterol (Beysen et al., 2012). More recently, it has also shown utility in the treatment of chronic idiopathic constipation (Khanna and Camilleri, 2021) by increasing bile acid concentrations entering the colon, thereby stimulating colonic secretion and motility (Bampton et al., 2002).

Linerixibat (GSK2330672) is an oral small molecule IBAT inhibitor, which has been shown to be effective in reducing PBC-associated pruritus and serum bile acids in two phase 2 studies (Hegade et al., 2017; Levy et al., 2020). Linerixibat was intentionally designed for minimal absorption from the GI tract (Wu et al., 2013). The rationale for this approach was to restrict drug exposure to the pharmacologic site of action while minimizing systemic exposure that may cause adverse events and drug–drug interactions (DDIs).

Preclinical studies demonstrated oral bioavailability of <1% in mice, rats, and dogs (Wu et al., 2013). However, in rodents this was caused by the low fraction absorbed from the GI tract, while in dogs the fraction absorbed was higher, consistent with relatively leakier canine intestines, and high hepatic extraction served as a secondary barrier to systemic exposure of linerixibat. While clinical trials have demonstrated minimal systemic linerixibat exposure in humans (Hegade et al., 2017; Ino et al., 2019), oral pharmacokinetics (PK) alone cannot prove that minimal GI absorption is the underlying cause. Understanding the basis of minimal oral systemic exposure is key in the context of linerixibat’s drug development. Studying both intravenous (IV) and oral linerixibat PK in humans enables quantification of the contributions of the fraction absorbed from the GI tract and first-pass hepatic extraction to the minimal systemic exposure. Thus, the objectives of this study were to assess the PK, absorption, metabolism, distribution and excretion of [<sup>14</sup>C]-linerixibat in humans following both IV and oral administration.

## Materials and Methods

### *Materials*

The [<sup>14</sup>C]-linerixibat (GSK2330672D) IV and oral solutions were obtained from Hammersmith Medicines Research Ltd (London, UK). Linerixibat (GSK2330672B) oral tablets were obtained from Wuxi Apptec Co., Ltd (Shanghai, China). Reference standards were provided by GlaxoSmithKline: GSK2330672B (free acid/free base, batch C13090301-QF17602, chemical purity 99.5%), [<sup>13</sup>C]-radiolabeled GSK2330672C (free acid/free base, batch R18284/139/2, radiochemical purity 97.8%) and [<sup>14</sup>C]-radiolabeled GSK2330672D (free acid/free base, batch DN27404-056A1, radiochemical purity 97.6%). All other solvents and reagents were of laboratory grade and purchased from commercial suppliers.

### *Study design*

This was a single-group, single-center, open-label, non-randomized, two-period, single-sequence [<sup>14</sup>C]-linerixibat mass balance study (Clinical trials.gov identifier [NCT03992014](https://clinicaltrials.gov/ct2/show/study/NCT03992014)) conducted at Hammersmith Medicines Research Centre (London, UK) between July 8, 2019 and August 26, 2019. The study adhered to the Declaration of Helsinki and was approved by the Health and Social Care Research Ethics Committee (National Health Service, UK).

Written informed consent was obtained from participants prior to any study-specific procedures. All analyses were performed according to a predefined protocol.

The study included a screening visit and two treatment periods separated by approximately 7 days ( $\geq 13$  days for oral doses) (**Figure 1**). During the treatment periods, participants resided in the unit from the morning of Day -1 (the day before dosing) until all procedures were completed at 168 h post-dose (Day 8). Participants were followed-up 1–2 weeks after the last assessment in Treatment Period 2.



All participants received a [<sup>14</sup>C]-linerixibat IV microtracer (100 µg; 3-h infusion) concomitant with 90 mg linerixibat oral tablets during Treatment Period 1 and a 90 mg [<sup>14</sup>C]-linerixibat oral solution during Treatment Period 2; all participants received their doses on site. The 90 mg oral dose of linerixibat was determined from a previous Phase 2a study (Hegade et al., 2017). The [<sup>14</sup>C]-linerixibat IV microtracer dosing regimen was extrapolated by allometry from preclinical *in vivo* data (see supplemental data) and *in vitro*-to-*in vivo* extrapolation (IVIVE) using data from *in vitro* analyses of human liver microsomes (presented in this paper). The relatively long (3 h) IV infusion of the relatively high microtracer dose (100 µg) was selected to ensure adequate characterization of the IV concentration-time curve considering projected rapid human elimination of IV dose (projected half-life 0.8 h, see supplemental data), as well as to reduce the maximal concentration expected at the end of IV infusion, to ensure it does not exceed established safety at the low concentrations achieved after oral linerixibat administration in previous human studies (Hegade et al., 2017).

For both treatment periods, participants underwent an overnight fast of at least 8 h. A blood sample was collected at 0 h, which was immediately followed by either a 90 mg oral dose of linerixibat administered as two 45 mg tablets along with a 3-h IV infusion of 100 µg of [<sup>14</sup>C]-linerixibat (Treatment Period 1) or a 90 mg [<sup>14</sup>C]-linerixibat dose administered as an oral solution (Treatment Period 2). Participants continued fasting for 2 h after dosing. The last blood sample was collected on Day 8 of Treatment Period 1 and on Day 7 (completing on Day 8) of Treatment Period 2. Urine and fecal samples were collected every 24 h for a total of 168 h after oral dosing for both treatment periods. Duodenal bile was collected on Day 1 of Treatment Period 1. For both treatment periods, the last vital sign assessment, electrocardiogram and brief physical examination were completed on Day 8.

Each participant received 9.25 kBq (250 nCi) in Treatment Period 1 and approximately 4.96 MBq (134.1  $\mu$ Ci) in Treatment Period 2. The total amount of radioactivity administered to each participant in the study was 4.97 MBq (134.3  $\mu$ Ci). It was estimated that the combined total effective dose for the two treatment periods would be <1 mSv. On this basis, the maximum administered activity complied with recommendation of 1 mSv maximum for Category IIa projects by International Commission on Radiological Protection (ICRP, 1992).

### ***Study population***

Healthy male participants between 30 and 55 years of age, with body weight  $\geq$ 50 kg, body mass index (BMI) 19–31 kg/m<sup>2</sup>, and a history of regular bowel movements were eligible for the study. Additional eligibility criteria: non-smokers or smokers who had not regularly smoked for 6 months prior to screening, no history of drug abuse and not exposed to significant radiation in the 3 years prior to the study. Standard participant criteria for a human radiolabeled disposition study were used (see **Supplementary methods** for full eligibility criteria).

### ***Sample collection and processing***

Blood samples were collected at pre-selected time points up to Day 7 after dosing and transferred into di-potassium ethylenediaminetetraacetic acid (EDTA) tubes. The maximum amount of blood collected from each participant over the duration of the study did not exceed 600 mL. Plasma was separated by centrifugation. Bile samples were collected via an Entero-test non-invasive string device (Neo-Medical Inc., Sparks, NV, USA) in Treatment Period 1 only. The Entero-test device was swallowed 3.5 h before the oral dose or start of the IV infusion to allow peristaltic transit to the duodenum. A food cue was used to stimulate gall bladder emptying at 2 h after the start of IV infusion, and the string was withdrawn 1 h later. All samples were stored frozen prior to shipment for analysis.

### ***Mass balance and excretion***

Total radioactivity excreted in urine and fecal samples was determined using liquid scintillation counting (LSC) and accelerator mass spectrometry (AMS) for Treatment Period 1 (Pharmaron ABS, Inc., Germantown, MD, USA) and by LSC for Treatment Period 2 (Covance Laboratories Limited, Harrogate, UK). For LSC, counting efficiency and quench correction were achieved automatically by an external standard ratio method. The lower limit of quantification (LLQ) for LSC was assigned as twice the mean background disintegration rate.

AMS instrument standards were analyzed alongside the samples for graphitization. For measurement of total radioactivity by AMS, urine and fecal samples underwent combustion (oxidation) and graphitization (reduction), and radioactivity was determined using a single stage accelerator mass spectrometer-250 (NEC, Middleton, WI, USA). For Treatment Period 1, the LLQ value using AMS for urine samples was 2.97 pg linerixibat equivalents per mL (Eq/mL) and for fecal samples was 17.2 pg Eq/mL. For the assessment of radioactivity in feces, one participant was a recovery outlier and was not included in the mean calculations.

### ***Pharmacokinetic assessments***

Plasma concentrations of [<sup>14</sup>C]-linerixibat (parent drug) were determined by liquid chromatography (LC) and AMS (LC+AMS) and plasma concentrations of [<sup>12</sup>C]-linerixibat were determined by LC with tandem mass spectrometry; the details of these assays are presented in **Supplementary Methods**.

Quality control (QC) samples were analyzed with each batch of study samples against separately prepared calibration standards. For measurement of samples containing linerixibat using LC-MS/MS, four concentrations were used. For measurement of samples containing

[<sup>14</sup>C]-linerixibat using LC+AMS, three concentrations were used. To pass acceptance, no more than one-third of the QC results were to deviate from the nominal concentration by >15% (LC-MS/MS) or >20% (LC+AMS), and ≥50% of the results from each QC concentration were required to be within 15% (LC-MS/MS) or 20% (LC+AMS) of nominal. The applicable analytical runs met all predefined run acceptance criteria.

The total radioactivity of plasma from Treatment Period 1 was determined with AMS and from Treatment Period 2 with LSC and/or AMS. For plasma total radioactivity measured by AMS, the mean LLQ was 16.1 pg Eq/mL for Treatment Period 1 and 25.2 pg Eq/mL for Treatment Period 2. Further details of bioanalytical methodologies are provided in the **Supplementary Methods**.

### *Quantification and characterization of metabolites*

To create pooled plasma samples at 1–3 h following Treatment Period 1, individual participant plasma samples were vortexed and equal volumes of 1-, 2- and 3-h samples were pooled across participants. Using an area under the plasma concentration-time curve (AUC) approach (Hamilton et al., 1981), individual participant plasma samples were vortexed and pooled across all participants for the preparation of plasma AUC<sub>0–12h</sub> samples following Treatment Period 2.

Individual participant urine (0–24 h) and homogenized fecal (0–120 h) samples from Treatment Period 1 were pooled based on a ratio of the total weight of the sample excreted at each time point and were made to represent ≥95% of the total excreted radioactivity across all participants (Penner et al., 2009). For Treatment Period 2, representative individual participant fecal samples were created according to excretion balance data; weighed aliquots of selected samples of feces (from time intervals containing >2% of the administered dose) were combined proportionally to total sample weight to give a single pooled representative

sample per participant. The pooled fecal samples were extracted once with acetonitrile containing 0.2% formic acid and once with methanol containing 0.2% formic acid. Combined extracts were dried down and reconstituted with methanol containing 10% dimethyl sulfoxide (DMSO). Samples were diluted with 0.1% formic acid in water prior to LC-MS/MS analysis with quantitation by microtiter plates scintillation counting from collected fractions. The total radioactivity of pooled Treatment Period 2 (oral) feces samples was expressed as a percentage of the total administered radioactive dose. Calculations were based on the percentage of administered dose and the pooled weight ratio data of the individual samples.

Cross-participant pools of all sample types (plasma AUC<sub>0-12h</sub>, urine [0-24 h] and fecal [0-120 h, Treatment Period 1] samples) were prepared after each individual participant pool was created by taking a constant proportion the participant pool. Pre-dose plasma, urine and fecal samples were created. These pools were used to obtain background values to subtract from the post-dose pools for analysis by AMS. Prior to analysis by LC+AMS, plasma samples were extracted with acetonitrile and then methanol containing 0.2% formic acid, reconstituted in 10% DMSO in methanol and diluted with 0.1% formic acid in water; fecal samples were extracted with acetonitrile containing 0.1% formic acid, then with methanol containing 0.2% formic acid (3X) and diluted with 0.1% formic acid in water; and urine was centrifuged. All the samples analyzed by AMS were spiked with [<sup>12</sup>C]-linerixibat reference standard.

Duodenal bile was first extracted with acetonitrile from the bile string samples, followed by a second extraction with water. Aliquots of the bile extracts were mixed with scintillation fluid and measured separately by LSC; vials were individually counted for 15 min. Following analysis and review of each sample, certain extracts were combined prior to further analysis by LC+AMS.

Radiochromatograms of plasma (Treatment Period 1 and 2) and urine, homogenized feces and bile string (Treatment Period 1) were generated by collecting LC fractions, followed by graphitization and analysis of fractions by AMS. Column recovery was calculated based on total amount of radioactivity ( $^{14}\text{C}$ ) injected and the amount recovered. Metabolites were quantified in plasma, urine, fecal (Treatment Period 1) and bile string extracts using AMS. Further details are provided in **the Supplementary Methods**.

Metabolite identification for the Treatment Period 2 feces was conducted using high-resolution mass spectrometry, where the chromatographic retention time, accurate mass, and  $\text{MS}^n$  fragmentation pattern were used for structure elucidation of metabolites. Metabolites and linerixibat assignments in the AMS radiochromatograms were made by retention time matching with either a corresponding linerixibat reference standard UV peak, metabolites from Treatment Period 2 feces or metabolite peaks from prior metabolite studies (unpublished data).

### ***Pharmacokinetics and statistical analysis***

No formal sample size calculation was performed for this study. As the objective was to gain a better understanding of the PK, excretory routes and metabolic profile of linerixibat, inclusion of 4–6 participants was deemed sufficient (Penner et al., 2009).

Plasma linerixibat, [ $^{14}\text{C}$ ]-linerixibat and total radioactivity concentration-time data were analyzed by non-compartmental methods with Phoenix<sup>®</sup> WinNonlin<sup>®</sup> Version 6.3 (Certara USA, Inc., Princeton, NJ) using the actual sampling times for derivation of PK parameters. Linerixibat plasma kinetics in terms of maximum observed plasma concentration ( $C_{\text{max}}$ ), time to  $C_{\text{max}}$ ,  $\text{AUC}_{0-t}$ ,  $\text{AUC}_{0-24}$  and  $\text{AUC}_{0-\text{inf}}$ , terminal phase rate constant, apparent terminal phase half-life, clearance, volume of distribution at steady-state, absolute bioavailability, fraction of

drug escaping first-pass hepatic clearance, hepatic extraction ratio, and fraction absorbed were calculated from plasma concentration-time data.

The hepatic plasma clearance ( $Cl_{h,iv,plasma}$ ) was calculated as the difference between linerixibat total plasma clearance ( $Cl_{iv}$ ) and renal clearance ( $Cl_{renal,iv}$ ). Hepatic blood clearance ( $Cl_{h,iv,blood}$ ) was determined by adjusting the hepatic plasma clearance by the linerixibat blood-to-plasma ratio of 0.678 determined *in vitro* (unpublished data). The hepatic extraction ratio ( $E_h$ ) was then determined by dividing  $Cl_{h,iv,blood}$  by hepatic blood flow ( $Q_h$ ) utilizing the literature reference value of 1660 mL/min (Edginton et al., 2006). The fraction of linerixibat that escapes first-pass liver extraction ( $F_h$ ) was determined as one minus the  $E_h$ . The fraction of linerixibat absorbed ( $f_a$ ), including the fraction escaping first-pass gut metabolism ( $f_g \sim 100\%$ ), was determined by dividing the absolute oral bioavailability ( $F$ ) by the  $F_h$ .

## Results

A total of 6 participants were enrolled in this study and all participants completed the study.

Mean age was 41.2 (standard deviation [SD] 8.1; range 33–53) years, and mean BMI was 24.0 (SD 1.1; range 22.9–26.0) kg/m<sup>2</sup>.

Linerixibat and total drug-related radioactivity in the systemic circulation rapidly attained steady-state during the 3-h IV infusion and declined rapidly at the end of the infusion with detectable concentrations at up to 6 h (**Figure 2**). The short elimination half-life (0.828 h) after IV administration was consistent with the high systemic clearance (61.8 L/h) and low volume of distribution (16.3 L) of linerixibat (**Table 1**). The exposure ratio of plasma linerixibat to plasma total radioactivity, as well as the metabolite profiling, showed that linerixibat was the predominant contributor to total radioactivity in the circulation (86% of total radioactivity in plasma), demonstrating linerixibat was minimally metabolized.

Elimination of IV radioactivity was 80% fecal and 20% renal, predominantly as unchanged parent drug (90–97% of fecal, bile string, and urinary radioactivity) (**Tables 1 and 2; Figure 3A**).

Standard *in vitro* studies identified oxidative metabolism in human liver microsomes as the most likely route of linerixibat clearance, which was mediated by cytochrome P450 (CYP) 3A4, but not the other major CYP enzymes (unpublished data). Scaled unbound intrinsic clearance of linerixibat in human liver microsomes was high (241 L/h) based on an *in vitro* incubation half-life of 39.4 min at 1 mg/mL microsomal protein concentration [microsome fraction unbound = 0.299; 39.7 mg microsomal protein/g of liver and 24.5 g of liver/kg body weight (Barter et al., 2007; Barter et al., 2008), assuming 70 kg human body weight) as described previously (Obach et al., 1997; Obach, 1999)]. This microsomal intrinsic clearance was extrapolated to human using IVIVE based on the well-stirred liver model, and the human



clearance was predicted to be 31 L/h [plasma fraction unbound = 0.245; blood-to-plasma ratio = 0.678; human hepatic blood flow = 96.6 L/h (Yang et al., 2007)]. This prediction was within 1.5-fold of the high hepatic clearance observed in this study (80% of human systemic elimination of IV dose).

The geometric mean half-life of linerixibat was approximately an order of magnitude longer after oral versus IV administration (6–7 h vs 0.8 h). Based on the observed shorter half-life after IV administration compared with oral administration, linerixibat displays absorption-limited, flip-flop kinetics; therefore, the terminal slope of the oral concentration-time profile (**Table 1**,  $\lambda_z$ ) reflects the oral absorption rate constant, which was estimated to be 0.1 h<sup>-1</sup>. Linerixibat exhibited minimal oral absorption (absolute oral bioavailability of 0.0517%) primarily due to the very low fraction absorbed (0.167%;  $f_g \sim 100\%$ , see Table 3 results below). High hepatic extraction (77.0%) acted as a secondary barrier to systemic exposure, accounting for the approximately 4-fold lower oral bioavailability versus fraction absorbed.  $T_{max}$  was highly variable after oral administration of both tablet and solution formulations with the large range overlapping across the two (**Table 1**).

As expected for a minimally absorbed drug, the oral [<sup>14</sup>C]-linerixibat dose was almost entirely excreted (>99% of the recovered radioactivity) in feces as unchanged and unabsorbed linerixibat. Three very minor oxidative metabolites, M8, M9, and M10 were detected, but they accounted for negligible radioactivity after correcting for co-eluting radiochemical impurity E and degradant K (**Table 3**), thus supporting  $f_g$  being approximately 100%. Consistent with low oral fraction absorbed, and approximately 80%/20% fecal/renal excretion of IV [<sup>14</sup>C]-linerixibat, urinary elimination of orally administered radioactivity was negligible (<0.04% of dose).

Following administration of oral [ $^{14}\text{C}$ ]-linerixibat solution, the exposure to total radioactivity was far higher and longer than to parent drug (**Figure 4**; individual participant data in **Supplementary Figure S1**). The geometric mean  $C_{\text{max}}$  and exposure ratios of plasma linerixibat to plasma total radioactivity showed that linerixibat represented only 2–6% of the total radioactivity in plasma. This low fraction of parent linerixibat accounting for oral plasma total radioactivity concentrations was conceptually inconsistent with parent linerixibat accounting for the majority of radioactivity administered via IV infusion. It also differs from previous profiling of human plasma obtained following 14 days of oral 90 mg twice daily non-radiolabeled linerixibat dosing, where only the parent drug, but no linerixibat-related metabolites were observed in circulation (Nunez et al., 2016). The low fraction of parent linerixibat accounting for plasma total radioactivity following oral dosing in Treatment Period 2 is likely an artifact of 2.4% radiochemical impurities for a drug with <0.2% fraction absorbed. Furthermore, these radiochemical impurities had a 70-fold higher specific activity than isotopically diluted [ $^{14}\text{C}$ ]-linerixibat in the oral dose formulation and were profiled using AMS, which provides a measure of total radiocarbon (see supplement for discussion of results presented in **Figure 5**).

## Discussion

Characterization of the human PK and metabolism of [<sup>14</sup>C]-linerixibat after both IV and oral administration is important in the context of a drug that has minimal systemic exposure and where the GI lumen is the site of pharmacology. Only by assessing both modes of administration can we quantify the contribution of fraction absorbed from the GI tract and of first-pass hepatic extraction to the minimal systemic exposure. The current study established that linerixibat exhibits minimal systemic exposure due to low intestinal absorption.

Furthermore, intestinal absorption was slow, resulting in flip-flop kinetics that were limited by absorption rate, as evidenced by a longer oral versus IV half-life (6–7 h vs 0.8 h).

Technically, fraction absorbed is  $f_a \times f_g$ , and, typically, direct calculation of  $f_g$  would have been possible based on oral and IV metabolite load data given that oral metabolite load = IV metabolite load + metabolites generated on first pass through the GI and the liver (Harrell et al., 2019). Unfortunately, the oral metabolite load calculation for linerixibat is not feasible since the major contributors to oral radioactivity exposure were radiochemical impurities and their metabolites. However, minimal first-pass gut wall metabolism ( $f_g \sim 100\%$ ) is substantiated by negligible recovery of metabolites in excreta after oral administration of [<sup>14</sup>C]-linerixibat (**Table 3**). Finally, there is no evidence that linerixibat accesses enterocytes and their metabolic enzymes due to exceedingly low passive permeability, and while linerixibat is taken up into hepatocytes by OATP1B, it is not transported by intestinal OATP2B1 (unpublished data).

These results have a direct impact on late-stage clinical development of linerixibat. *In vitro* screening of both perpetrator and victim DDI risks flagged linerixibat as both a substrate and inhibitor of OATP1B1 (half maximal inhibitory concentration [ $IC_{50}$ ] = 2.69  $\mu$ M), OATP1B3 ( $IC_{50}$  = 0.265  $\mu$ M) and CYP3A4 ( $K_I$  = 1.3 mM,  $k_{inact}$  = 9.6  $h^{-1}$ ,  $f_{u,inc}$  = 0.3) (unpublished data).

Based on PK results from this study and current regulatory guidelines, linerixibat's potential as a perpetrator or victim of DDIs via CYP3A4 and OATP1B does not meet the criteria for clinical evaluation (US FDA, 2020a).

Although linerixibat is an OATP1B1 and OATP1B3 substrate *in vitro*, the present study clearly established minimal intestinal absorption as the basis of minimal systemic exposure of linerixibat, with the liver acting as a secondary barrier to systemic exposure with a hepatic extraction of 77.0%. Assuming complete inhibition of hepatic OATP1B, oral bioavailability would increase at the most from 0.05% to the fraction absorbed of <0.2%. Consequently, systemic exposure would increase at most only by approximately 4-fold, which would be well within systemic safety margins. Due to linerixibat exhibiting flip-flop kinetics limited by absorption rate, potential inhibition of hepatic clearance would not increase exposure following oral dosing because absorption is approximately 10-fold slower than elimination. As such, only the hepatic first-pass effect is relevant to victim DDI potential.

Based on the results of the present study, further clinical studies to assess the effect of renal impairment are not scientifically justified. The systemic exposure of linerixibat after oral administration is not expected to be impacted by renal impairment or by a reduction in hepatic clearance due to circulation of elevated levels of uremic toxins due to its minimal oral absorption, negligible dose recovery in urine after oral administration and flip-flop kinetics.

Although linerixibat is a CYP3A4 substrate *in vitro*, inhibition of metabolic clearance by a co-administered CYP3A4 inhibitor is not expected to impact linerixibat disposition or systemic PK. This is because orally administered linerixibat was eliminated almost entirely as unabsorbed and unchanged parent drug in feces. About one in 2000 orally administered linerixibat molecules that do enter the systemic circulation are eliminated as unchanged drug in bile/feces (approximately 80%) and urine (approximately ~20%).

Intrinsic clearance of linerixibat in human liver microsomes was high (241 L/h) with metabolism exclusively via CYP3A4 and no other major CYP isoforms (unpublished observations). IVIVE based on the well-stirred liver model predicted a human hepatic clearance of 31 L/h, which was within 1.5-fold of the observed human hepatic clearance (80% of systemic clearance). Therefore, it was surprising that human clearance occurred primarily by direct excretion of parent drug with negligible metabolism. Even following a thorough literature review, it appears that linerixibat is the only reported example of this phenomenon, which may be related to the unusual physicochemical properties needed to achieve minimal absorption (Wu *et al.*, 2013). An interesting future direction may be to determine whether a more complex *in vitro* hepatocyte system that re-establishes bile canaliculi, while maintaining CYP metabolic activity, is capable of describing *in vivo* hepatobiliary disposition of linerixibat.

Biliary excretion was studied in bile duct-cannulated male Han Wistar rats and male Beagle dogs following oral administration of [<sup>14</sup>C]-linerixibat (unpublished data). The original conclusion of these studies was that fecal excretion was the major elimination pathway for oral [<sup>14</sup>C]-linerixibat (rat = 93.9% and dog = 82.0% of dosed radioactivity), while biliary and urinary excretion represented minor elimination routes (rat bile = 3.0% and urine = 0.1%; dog bile = 6.7% and urine = 2.7% of dosed radioactivity). The outcome of the present clinical study resulted in re-examination of preclinical oral [<sup>14</sup>C]-linerixibat mass balance studies, assuming radioactivity recovered in bile and urine represents absorbed radioactivity. Upon re-examination of these preclinical results in terms of elimination of absorbed radioactivity, it becomes evident that biliary excretion of unchanged linerixibat is also the major route of elimination in rats and dogs. Biliary/urinary recovery of absorbed radioactivity in rats and dogs was 97%/3% and 71%/29%, respectively, which is consistent with ~80%/20% linerixibat-related material excretion in humans. Rat and dog biliary radioactivity consisted

primarily of parent linerixibat 73% and 75%, mono-oxygenated metabolites  $\leq 6.5\%$  and 19.5%, respectively, and radiochemical impurity-related radioactivity  $\geq 13\%$  and 3.4%, respectively. Mechanistically, the first step in linerixibat biliary excretion is mediated by OATP1B1 and OATP1B3 hepatic uptake (unpublished data); subsequent canalicular secretion is not mediated by P-gp or BCRP (unpublished data), leaving multidrug resistance associated protein (MRP)2 as the most likely canalicular transporter (Patel et al., 2019), but one which is not studied during drug development due to lack of clinical evidence supporting involvement of MRP2 in clinical DDIs (US FDA, 2020a).

Typically, in the development of small molecule drugs, the primary purpose of a radiolabeled study is the characterization of human *in vivo* metabolism to ensure that preclinical rodent and non-rodent toxicology species provide adequate coverage of the major human metabolites (US FDA, 2020b). After IV administration, unchanged linerixibat was the predominant form in human circulation and excreta (**Table 2, Figure 2**). This was consistent with non-radiolabeled metabolite profiling of human plasma obtained after 90 mg oral linerixibat dosed twice daily for 2 weeks (Nunez et al., 2016). However, the plasma radiochromatogram (derived by AMS) after oral administration was complex (**Figure 5**). As described in Results, the observed complexity can be attributed to 2.4% of radiochemical impurities, with an estimated 70-fold higher specific activity than the isotopically-diluted parent drug, which exhibits low oral absorption ( $<0.2\%$ ). Despite this complex finding, the overall metabolite profile supports the absence of major human metabolites. AMS radiochromatogram of cross-participant plasma pool extract ( $AUC_{0-12\text{ h}}$ ) following oral administration of 90-mg oral solution of [ $^{14}\text{C}$ ]-linerixibat is described in detail in the **Supplementary Results** and depicted in **Figure 5**.

In conclusion, the present two-period clinical study of [ $^{14}\text{C}$ ]-linerixibat IV and oral PK and disposition established linerixibat as a minimally absorbed drug, which after oral

administration is almost entirely recovered as unabsorbed and unchanged parent drug in feces. This study supports that, following oral administration, linerixibat is effectively restricted to the GI lumen, the site of pharmacology for IBAT inhibition. Additionally, this study demonstrated flip-flop oral absorption-rate-limited systemic PK. These results have considerable impact on late-stage clinical pharmacology studies as discussed above. Finally, linerixibat presents a fascinating novel case study, where human clearance by CYP3A4 metabolism was quantitatively predicted to be high, but *in vivo* metabolism was found to be minimal, with clearance predominantly by direct biliary/fecal excretion of the unchanged parent drug.

## **Acknowledgments**

Editorial support (in the form of writing assistance, including preparation of the draft manuscript under the direction and guidance of the authors, collating and incorporating authors' comments for each draft, assembling tables and figures, grammatical editing and referencing) was provided by Mary E Morgan, PhD, and Hayley S Butler, PhD, of Fishawack Indicia Ltd., part of Fishawack Health, and funded by GlaxoSmithKline.



### **Authorship contribution**

Participated in research design: MJZ-G, GCY, DK, JM, and RLO-S.

Conducted experiments: DAB, KMT, AIP, JMTJD, MS, and LC.

Contributed new reagents and analytic tools: Not applicable.

Performed data analysis: MJZ-G, GCY, DK, DAB, JLP, KMT, AIP, MS, LC, MA, MMM, BS, and RLO-S.

Wrote or contributed to the writing of the manuscript: All authors.

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## Footnotes

This study was funded by GlaxoSmithKline (Study 205895).

MJZ-G, DK, DAB, AIP, JM, JLP, KMT, MMM, MA, BS, and GCY are GSK employees and hold GSK shares. MS is an employee of Pharmaron US. LC is an employee of Covance Laboratories Limited, UK. RLO-S and JD do not have any conflicts of interest.

Information on GSK's data sharing commitments and requesting access can be found at:

<https://www.clinicalstudydatarequest.com>

The data presented in this manuscript was presented in part at the American Association for the Study of Liver Diseases November 13–16, 2020 (Zamek-Gliszczyński et al. *AASLD 2020*; Poster 1257).

## Legends for Figures

### Figure 1: Study schematic

### Figure 2: Parent [<sup>14</sup>C]-linerixibat and total drug-related radioactivity concentration-time profiles following 100 µg IV microdose of [<sup>14</sup>C]-linerixibat (n=6)

The IV microdose of [<sup>14</sup>C]-linerixibat (infused over 3 hours) was administered concomitantly with a non-radiolabeled 90 mg tablet oral dose.

IV, intravenous.

### Figure 3: Fecal, urinary, and total recovery of radioactivity, following administration of (A) an IV [<sup>14</sup>C]-linerixibat microtracer over 3 hours (n=6, mean ± SD) and (B) a 90 mg [<sup>14</sup>C]-linerixibat oral solution (n=5<sup>a</sup>, mean ± SD)

<sup>a</sup>n=5 for all time points as one participant's recovery was only 20.8% of the dose and considered anomalous compared with the 97% recovery in the other 5 participants (outlier by Q test; no fecal samples on study days 2 and 6 when the other 5 participants produced daily fecal samples; prune juice given from day 6 to elicit bowel movements, resulting in feces with no detectable radioactivity from day 7 onwards).

SD, standard deviation.

### Figure 4: Parent linerixibat and total drug-related radioactivity concentration-time profiles following 90-mg oral solution of [<sup>14</sup>C]-linerixibat (n=6)

Individual participant data shown in **Supplementary Figure S1**.

LLQ, lower limit of quantification.

### Figure 5: AMS radiochromatogram of cross-participant plasma pool extract (AUC<sub>0-12 h</sub>) following oral administration of 90-mg oral solution of [<sup>14</sup>C]-linerixibat.

AMS, accelerator mass spectrometry; AUC, area under the plasma concentration-time curve; DPM, disintegrations per minute; ROI, region of interest.



## Tables

**Table 1:** Summary of pharmacokinetic parameters for linerixibat (n=6, unless stated otherwise)

PK parameter (units)	Summary statistics	90 mg linerixibat oral tablets	100 µg [ <sup>14</sup> C]-linerixibat IV infusion	90 mg [ <sup>14</sup> C]-linerixibat oral solution
C <sub>max</sub> (pg/mL)	Geometric mean (CVb%)	120 (108)	638 (27.3)	158 (270)
	95% CI	(47.8, 302)	(481, 845)	(34.3, 726)
t <sub>max</sub> (h)	Median	2.25	1.49	7.50
	Range	(0.500, 5.50)	(0.983, 2.50)	(2.00, 48.1)
AUC <sub>0-t</sub> (h•pg/mL)	Geometric mean (CVb%)	749 (125)	1560 (26.8)	1040 (79.4)
	95% CI	(270, 2070)	(1190, 2060)	(501, 2170)
AUC <sub>0-inf</sub> (h•pg/mL)	n	3	6	3
	Geometric mean (CVb%)	1550 (25.8)	1570 (26.7)	1630 (95.3)
	95% CI	(826, 2920)	(1190, 2070)	(222, 12000)
λ <sub>z</sub> (h <sup>-1</sup> )	Geometric mean (CVb%)	0.102 (124)	0.836 (18.26)	0.110 (64.3)
	95% CI	(0.009, 1.13)	(0.692, 1.01)	(0.026, 0.479)
t <sub>1/2</sub> (h)	n	3	6	3
	Geometric mean (CVb%)	6.76 (124)	0.828 (18.3)	6.25 (64.3)
	95% CI	(0.614, 74.4)	(0.685, 1.00)	(1.45, 27.0)
V <sub>ss</sub> (L)	Geometric mean (CVb%)	NA	16.3 (35.7)	NA
	95% CI	NA	(11.4, 23.5)	NA
Cl <sub>iv,plasma</sub> (mL/min)	Geometric mean (CVb%)	NA	1030 (27.3)	NA
	95% CI	NA	(779, 1370)	NA
Cl <sub>R,iv,plasma</sub> (mL/min)	Geometric mean (CVb%)	NA	163 (37.2)	NA
	95% CI	NA	(112, 238)	NA
Cl <sub>h,iv,plasma</sub> (mL/min)	Geometric mean (CVb%)	NA	867 (26.4)	NA
	95% CI	NA	(660, 1140)	NA
Cl <sub>h,iv,blood</sub> (mL/min)	Geometric mean (CVb%)	NA	1280 (26.4)	NA
	95% CI	NA	(974, 1680)	NA
E <sub>h</sub>	Geometric mean (CVb%)	NA	0.770 (26.4)	NA

	95% CI	NA	(0.586, 1.01)	NA
F <sub>h</sub>	Geometric mean (CVb%)	NA	0.244 (68.4)	NA
	95% CI	NA	(0.113, 0.527)	NA
fa*	Geometric mean (CVb%)	0.00167 (73.7)		NA
	95% CI	(0.000739, 0.00380)		NA
F	Geometric mean (CVb%)	0.000517 (120)		NA
	95% CI	(0.000192, 0.00140)		NA

AUC<sub>0-t</sub>, area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a participant across all treatments; AUC<sub>0-inf</sub>, area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time;  $\lambda_z$ , elimination rate constant; CI, confidence interval; Cl<sub>h,iv,plasma</sub>, hepatic clearance from plasma; Cl<sub>h,iv,blood</sub>, hepatic clearance from blood; CL<sub>R,iv,plasma</sub>, renal clearance; C<sub>max</sub>, maximum observed plasma concentration; CVb, between participant variability; E<sub>h</sub>, hepatic extraction ratio; F<sub>h</sub>, fraction of linerixibat that escapes first pass liver extraction; fa, fraction of linerixibat absorbed; F, absolute oral bioavailability for linerixibat; n, number of participants with available data to estimate the PK parameter; NA, not assessed; PK, pharmacokinetic; t<sub>1/2</sub>, half-life; t<sub>max</sub>, time of occurrence of C<sub>max</sub>; V<sub>ss</sub>, volume of distribution.

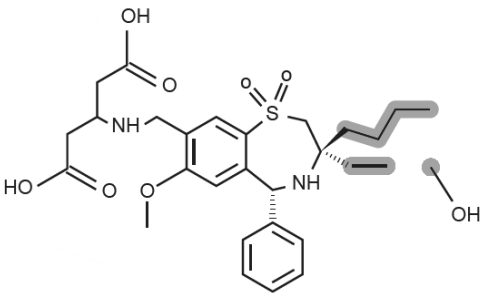
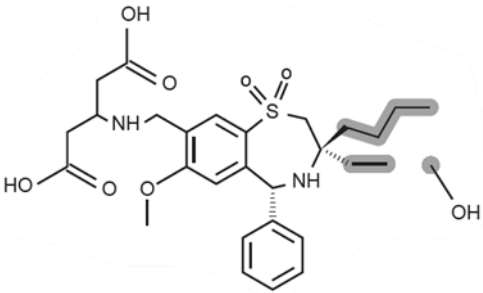
\*fa includes the fraction escaping first-pass gut metabolism (fg~100%)

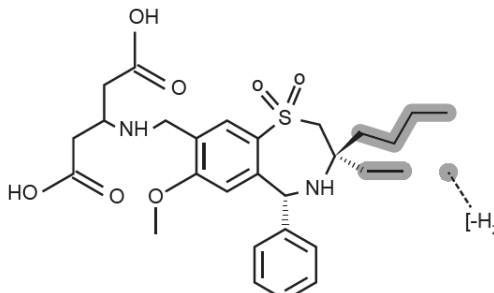
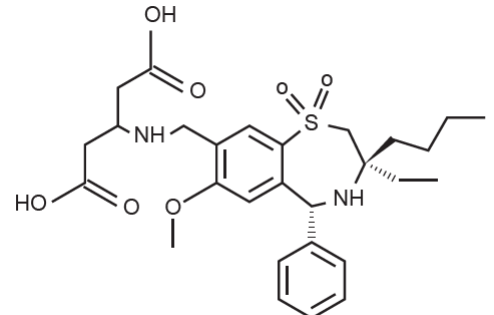
**Table 2:** [<sup>14</sup>C]-linerixibat-related radioactivity following intravenous infusion of radiolabeled microdose.

Component	Nominal retention time (min)	% radioactivity			
		Plasma	Urine	Bile	Feces
Impurity E	35.2	0.68	1.46	1.13	NA
Linerixibat	36.4	85.64	97.28	96.22	90.57
P3*	42.0	1.24	NA	NA	NA
P4*	47.4	1.78	NA	NA	NA

NA, not assessed. \*Identity unknown.

**Table 3:** Mean quantification of the radioactive components in fecal extracts following a single oral dose of [<sup>14</sup>C]-linerixibat at 90 mg (134.1 μCi) using offline analysis<sup>a</sup>

Metabolite ID	Metabolite structure	Radioactivity in feces, mean (%) of administered dose <sup>a</sup>	Radioactivity in spiked feces, %
M8 <sup>b</sup>	 <p style="text-align: center;">Oxidation</p>	1.08 (1.03)	ND
K <sup>b</sup>	Unassigned		1.05
M9	 <p style="text-align: center;">Oxidation</p>	0.14 (0.14)	ND

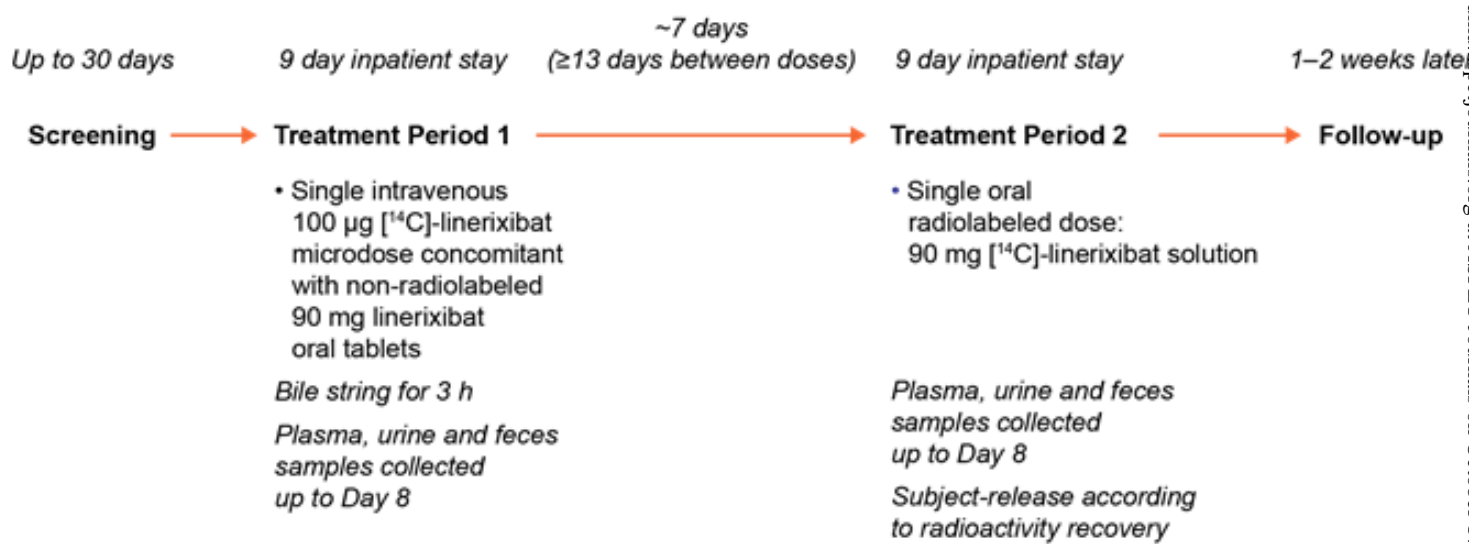
M10 <sup>b</sup>	 <p style="text-align: center;">Dehydrogenation (2 isomers)</p>	1.32 (1.27)	ND
E <sup>b</sup>			1.58
Linerixibat (parent)		93.9 (90.5)	97.4
Total quantified		96.5 (93.0)	100
Dose in sample analyzed, %		93.2	NA
Dose in total sample, %		96.4	NA

ND, not determined.

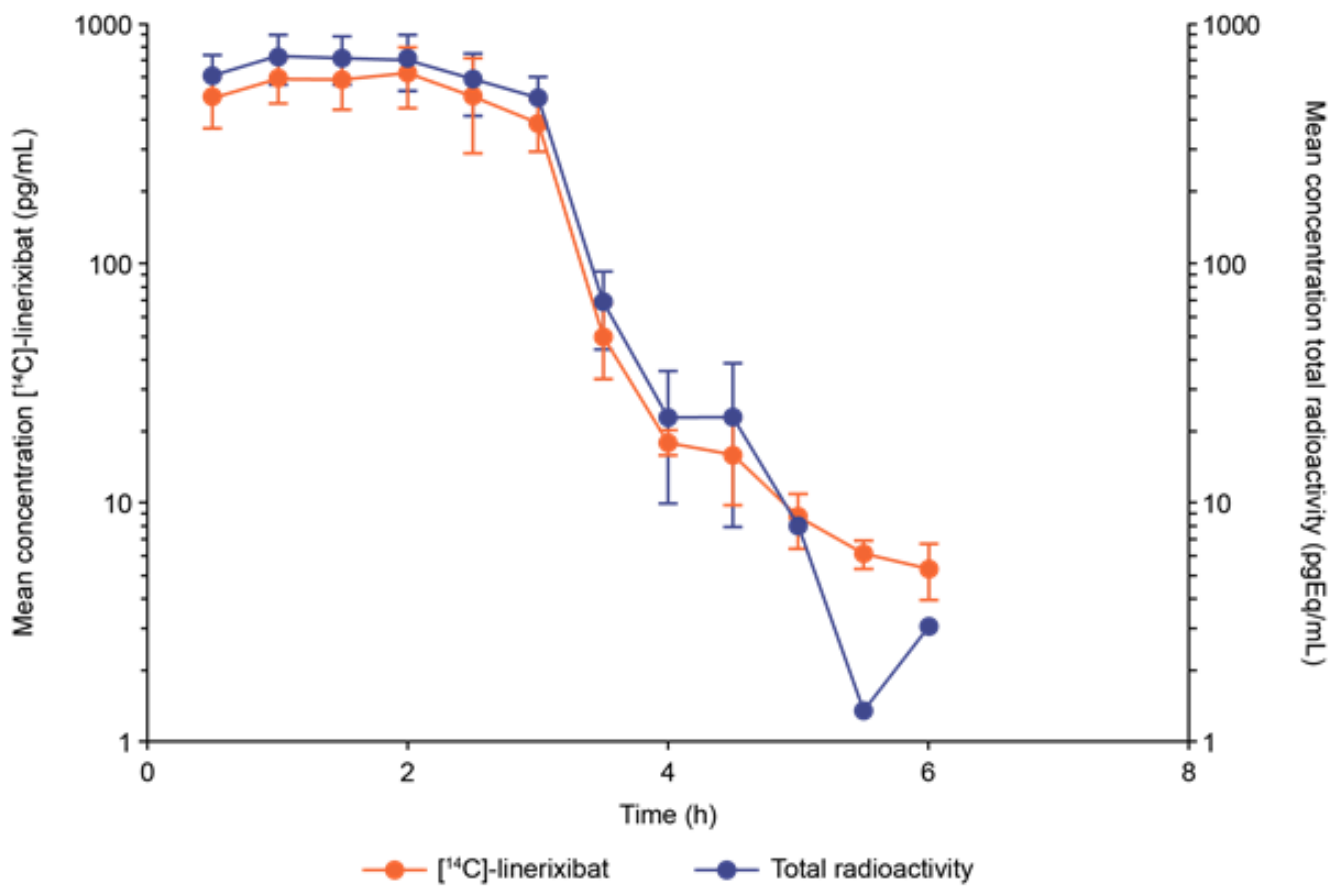
<sup>a</sup>n=5; a single participant was a recovery outlier and was not included in the mean calculations.

<sup>b</sup>Based on further calculations, it was determined that the majority of the radioactivity of these peaks were from degradant K and impurity E.

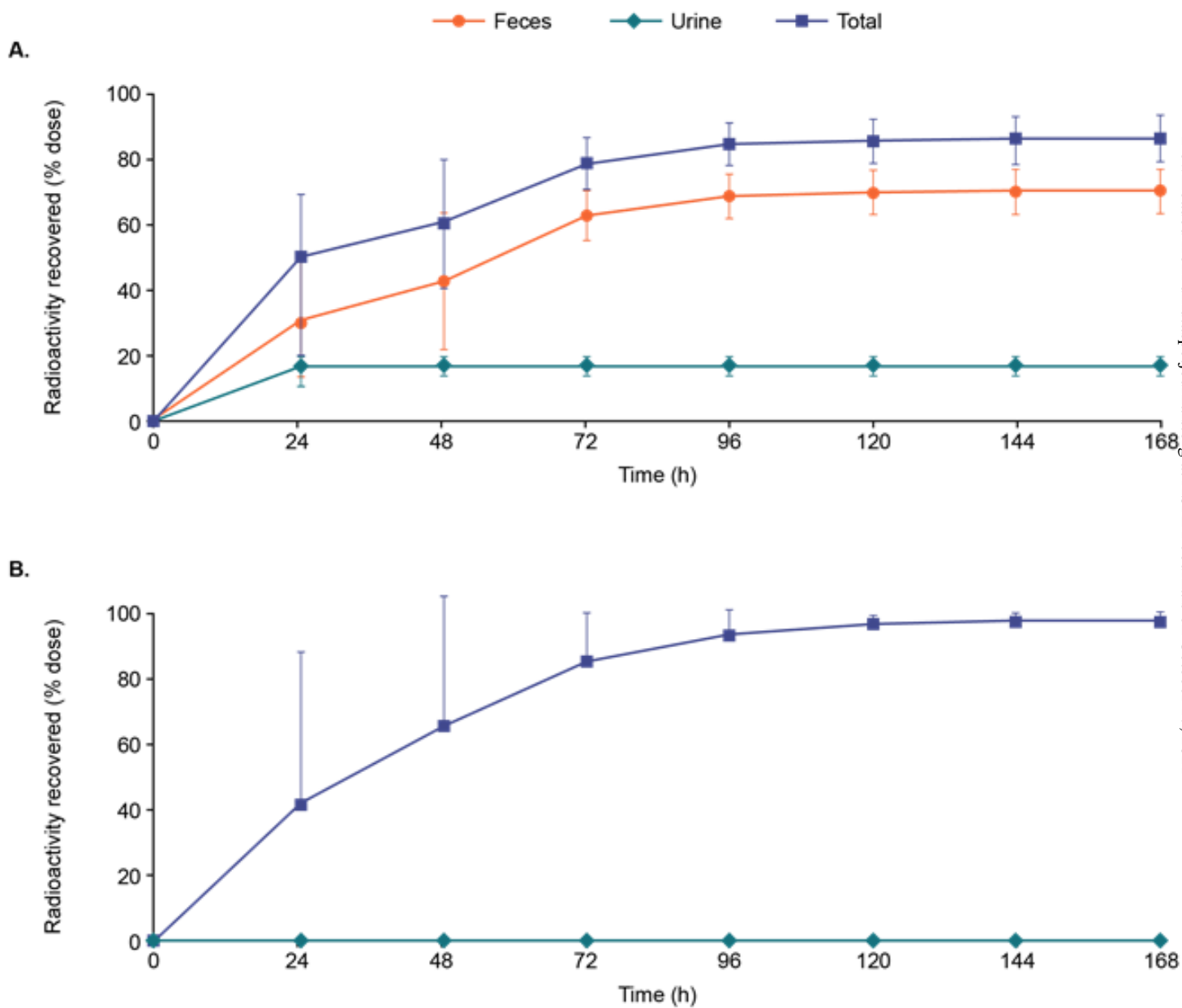
# Figure 1



# Figure 2

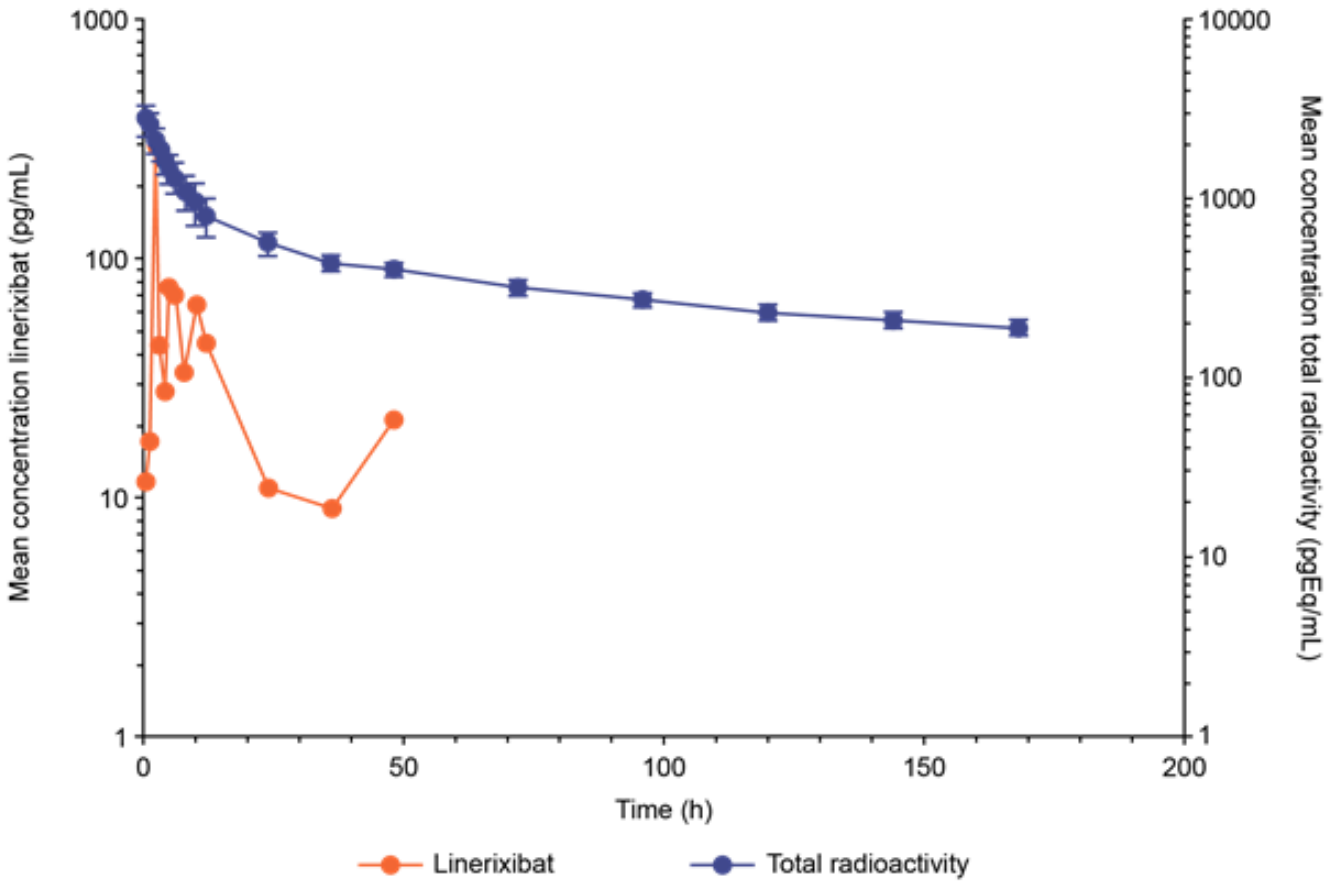


# Figure 3





# Figure 4



# Figure 5

