Title: Metabolism and Disposition of the Hepatitis C Protease Inhibitor Paritaprevir in Humans

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Running Title

a) Mass balance, metabolism and excretion of Carbon-14 radiolabeled Paritaprevir in humans.

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HCV, hepatitis C virus; DAAs, direct-acting antiviral agents; SVR, sustained virologic response;
AUC, area under the curve; CYP, cytochrome P450; CID, collisional induced dissociation; LSC, liquid scintillation counting; HPLC, high-performance liquid chromatography; SPE, solid phase extraction, ACN, acetonitrile; FaSSIF, fasted state simulated intestinal fluid; FeSSIF, fed state simulated intestinal fluid; ABT-450, paritaprevir; paritaprevir/r, paritaprevir/ritonavir; ABT-267, ombitasvir; ABT-333, dasabuvir; 1-ABT, 1-aminobenzotriazole.
Abstract

Paritaprevir (also known as ABT-450), a potent NS3-4A serine protease inhibitor (identified by AbbVie and Enanta) of the hepatitis C virus (HCV), has been developed in combination with ombitasvir and dasabuvir in a three direct-acting antiviral oral regimen (DAAs) for the treatment of patients infected with HCV genotype 1. This article describes the mass balance, metabolism and disposition of paritaprevir in humans. Following the administration of a single 200-mg oral dose of [14C]paritaprevir co-administrated with 100-mg ritonavir to four male healthy volunteers, the mean total percentage of administered radioactive dose recovered was 96.5%, with recovery in individual subjects ranging from 96.0 to 96.9%. Radioactivity derived from [14C]paritaprevir was primarily eliminated in feces (87.8% of dose). Radioactivity recovered in urine accounted for 8.8% of dose. The biotransformation of paritaprevir in human involves 1) CYP-mediated oxidation on the olefinic linker, the phenanthridine group, the methylpyrazinyl group, or combinations thereof, and 2) amide hydrolysis at the acyl cyclopropane-sulfonamide moiety and the pyrazine-2-carboxamide moiety. Paritaprevir was the major component in plasma (90.1% of total radioactivity in plasma, AUC0-12h pool). Five minor metabolites were identified in plasma, including the metabolite M2, M29, M3, M13 and M6; none of the metabolites accounted for greater than 10% of the total radioactivity. Paritaprevir was primarily eliminated through the biliary-fecal route followed by microflora-mediated sulfonamide hydrolysis to M29 as a major component in feces (approximately 60% of dose). In summary, the biotransformation and clearance pathways of paritaprevir were characterized, and the structures of metabolites in circulation and excreta were elucidated.
Introduction

Hepatitis C virus (HCV) is a virus that is a common cause of viral hepatitis. Untreated HCV can lead to chronic liver disease, including cirrhosis, organ failure and liver cancer. An estimated 150-170 million people worldwide are chronically infected with HCV and have an increased risk of developing liver cirrhosis or liver cancer (Lavanchy, 2011; Mohd Hanafiah et al., 2013). Genotype 1 is the most prevalent genotype in the United States, Europe, and Japan, and globally accounts for approximately 60% of HCV infections (Zein, 2000).

HCV is an enveloped positive-strand RNA virus that replicates primarily in the cytoplasm of hepatocytes. Viral NS3-4A serine protease is a non-covalent heterodimer consisting of a catalytic subunit (the N-terminal one-third of NS3 protein) and an activating cofactor (NS4A protein), which is responsible for cleavage of the HCV polyprotein at four sites. HCV NS3-4A protease is essential for the viral replication process (Lin, 2006; Moradpour et al., 2007) and is a validated drug target (McHutchison et al., 2009; Poordad et al., 2011). Paritaprevir (ABT-450) is a potent macrocyclic noncovalent peptidomimetic inhibitor (identified by AbbVie and Enanta) of HCV NS3/4A protease, with 50% effective concentration values of 1.0, 0.21, 5.3, 19, 0.09, and 0.69 nM against stable HCV replicons with NS3 protease from genotypes 1a, 1b, 2a, 3a, 4a, and 6a, respectively (Pilot-Matias et al., 2015). Paritaprevir with ritonavir demonstrated robust in vivo responses with mean maximum decreases in HCV RNA from 3.89 to 4.11 log10 IU/mL following 3-day monotherapy in treatment-naïve HCV genotype-1 infected subjects among three dose groups (Lawitz et al., 2010). Paritaprevir with ritonavir has been used in combination with the NS5A inhibitor ombitasvir (formerly known as ABT-267), the nonnucleoside NS5B polymerase inhibitor dasabuvir (formerly known as ABT-333) for interferon-free direct-acting antiviral agents (DAA) treatment of HCV infection with or without ribavirin (Feld et al., 2014;
Kowdley et al., 2014; Zeuzem et al., 2014). Treatment with paritaprevir/r–ombitasvir and dasabuvir with ribavirin showed higher rates of sustained virologic response among patients with genotype 1a infection compared to that in the ribavirin-free group (Ferenci et al., 2014).

Paritaprevir is metabolized primarily by cytochrome P450 CYP3A4 (Bernstein et al., 2009). To mitigate the high first-pass and hepatic elimination, paritaprevir is co-administered with a low dose of ritonavir, a potent CYP and efflux transport inhibitor, as a boosting agent to improve systemic exposure. Clinical results confirmed that co-administration of ritonavir increases paritaprevir plasma levels and half-life, permitting once-daily dosing (Menon et al., 2009). A 100-mg ritonavir dose was found to be optimal for increasing paritaprevir exposure without negatively impacting the safety profile (Menon et al., 2015). The pharmacokinetics of paritaprevir increased in a greater than dose-proportional fashion and showed a 2-3 fold accumulation following multiple dosing (Bernstein et al., 2009). Paritaprevir was shown to be safe, well tolerated with minimal side effects in genotype 1 HCV-infected treatment-naïve subjects (Lawitz et al., 2010). This report describes the metabolism and disposition of a single 200-mg oral dose of [14C] paritaprevir, co-administered with single 100-mg oral dose of ritonavir, in healthy human subjects. The purpose of this study was to determine the mass balance, elucidate the routes and rates of excretion, and determine the urine and fecal recovery of radioactivity. The study also intended to identify the circulating metabolites in human plasma, elucidate the metabolite structures, determine the metabolite profiles in excreta and understand the metabolic pathway of paritaprevir in humans. Mechanistic assessment of the formation of paritaprevir metabolites in vitro was also conducted and discussed.

Materials and Methods

Drugs and Reagents
Paritaprevir ((2R,6S,12Z,13aS,14aR,16aS)-N-(cyclopropylsulfonyl)-6-[(5-methylpyrazin-2-yl)carbonyl]amino]-5,16-dioxo-2-(phenanthridin-6-yloxy)-1,2,3,6,7,8,9,10,11,13a,14,15,16,16a-tetradecahydrocyclopropa[e]pyrrolo[1,2-a][1,4]diazacyclopentadecine-14a(5H)carboxamidehydrate), [14C]paritaprevir and metabolite M2 reference material were supplied by Process Chemistry, AbbVie, Inc (North Chicago, IL). The chemical structure of paritaprevir is shown in Fig 1, (*) denotes the [14C]label position. The radiochemical synthesis of [14C]paritaprevir was conducted in three steps using [14C] potassium cyanide as the starting material. Radiochemical purity was >99% after HPLC purification. 5-Methylpyrazine 2-carboxylic acid (M13) was obtained from Sigma-Aldrich. All these reference standards were used as HPLC and mass spectrometric standards for structure confirmation.

Clinical Study

The clinical study was conducted at Covance Laboratories Inc., in conjunction with the Covance Clinical Research Unit (Madison, WI). In this open-label study, a total of four adult male subjects (N = 4) in general good health were selected to participate in the study according to the selection criteria. On the morning of Study Day 1, subjects received a single oral dose of [14C]paritaprevir and ritonavir under non-fasting conditions. The study drug, paritaprevir (200 mg active, 100 μCi [14C]) and ritonavir, 100 mg was administered as four (4 × 50 mg) hard gelatin capsules of paritaprevir and one capsule of ritonavir (100 mg). The total amount of liquid taken was approximately 240 mL, 30 minutes after starting a standardized breakfast. Subjects were confined to the study site for a minimum of 120 hours, post-dose, or up to a maximum of 312 hours, post-dose.
Blood samples were collected by venipuncture into potassium EDTA vacutainer collection tubes at the following times: 0 hour (predose), 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192 hours post dose. Plasma was separated via centrifugation and stored at –70°C.

Urine samples were collected over the following intervals: 0 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192 hours post dose. The aliquots of urine were frozen and maintained at –20°C prior to metabolite profiling.

Fecal samples were collected pre-dose and over the following intervals after dosing: 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, 144 to 168, 168 to 192 hours. All feces collected during a collection interval were kept frozen at –20°C prior to metabolite profiling.

**Total Radioactivity Measurement by Liquid Scintillation Counting**

All sample combustions were conducted using a Model 307 Sample Oxidizer (Packard Instrument Company) and the resulting $^{14}$CO$_2$ was trapped in a mixture of Perma Fluor and Carbo Sorb. The oxidation efficiency was evaluated daily of sample combustion by analyzing a commercial radiolabeled standard both directly in scintillation cocktail and by oxidation. Acceptance criteria were defined as combustion recoveries of 95 to 105%. Ultima Gold XR scintillation cocktail was used for samples analyzed directly. All samples were analyzed for radioactivity in Model 2900TR liquid scintillation counters (Packard Instrument Company) for at least 5 minutes or 100,000 counts. Each sample was homogenized or mixed with scintillation cocktail before radioanalysis. All samples were analyzed in duplicate if sample size allowed. If results from sample replicates (calculated as $^{14}$C dpm/g sample) differed by more than 10% from the mean value and sample aliquots had radioactivity greater than 200 dpm, the sample was rehomogenized and reanalyzed.
Blood samples were mixed and duplicate weighed aliquots (approximately 0.2 g) were combusted and analyzed by liquid scintillation counting (LSC). The representative lower limit of quantitation for blood was 92.1 ng equivalents/g. Plasma samples were mixed and duplicate weighed aliquots (approximately 0.2 g) were analyzed directly by LSC. The representative lower limit of quantitation for plasma was 71.7 ng equivalents/g. The urine samples were mixed and duplicate weighed aliquots (approximately 0.2 g) were analyzed directly by LSC. The representative lower limit of quantitation for urine was 71.7 ng equivalents/g. Fecal samples were combined across subjects at 24-hour intervals and the weight of each combined sample was recorded. A weighed amount of water was added and the sample was mixed. The sample was removed from the freezer and homogenized, or immediately homogenized using a probe-type homogenizer. Duplicate weighed aliquots (approximately 0.2 g) were combusted and analyzed by LSC.

**Sample Preparation for Metabolite Profiling**

Plasma samples were thawed at the room temperature and pooled across subjects at selected time points or using the Hamilton method (also known as time-point weighted AUC pool) (Hamilton et al., 1981) for each subject. Plasma samples were processed using a two-step extraction method. In brief, pooled plasma was extracted with a two-fold volume of acetonitrile (ACN):methanol (3:1, v/v), followed by sonication and vortexing. The quenched sample was centrifuged at 3000 rpm (2465 x g) for 15 min at 4°C. After collecting the supernatant, the protein pellets were extracted using 3-fold volume of ACN:methanol (3:1, v/v), with vortexing and sonication. After centrifugation at 3000 rpm (2465 x g) for 15 min at 4°C, the supernatant was collected and combined with the previous extract. Combined supernatants from the extracts were completely evaporated under a nitrogen stream at room temperature. The residues were
dissolved in 5 mL ACN:methanol (3:1, v/v) for solid phase extraction (SPE) using the Varian MEGA BE-C8 Bond Elut (Part# 12256002). The cartridge was conditioned using 2 x 5 mL ACN:methanol (3:1, v/v) and 2 x 5 mL 0.1% formic acid in water. The cartridge was pre-filled with 4.5 mL of deionized water with the stopcock closed and 0.5 mL of the extracted sample was loaded, mixing well before opening the stopcock to initiate the flow through the cartridge. Once the sample was loaded, the process was repeated until all the extracted sample was loaded to the column. The cartridge was washed with 5 mL deionized water and the sample was eluted with 3 x 5 mL ACN:methanol (3:1, v/v). The eluent was evaporated to dryness under a nitrogen stream at room temperature, and residue was reconstituted in 75 µL ACN:methanol (3:1, v/v), diluted with 150 µL of water before HPLC-MS-radiochemical detection analysis. An aliquot of the reconstituted sample was subjected to LSC counting to determine the total radioactivity recovery. The mean radioactivity recovery in the processed plasma samples was about 83%.

Pooled urine was extracted using SPE. In brief, SPE cartridge (Phenomenex Strata X-AW 33 µ Polymeric Weak Anion, 1 g/12 mL, Part# 8B-S038-HDG) was conditioned with 15 mL methanol and 15 mL deionized water. Aliquots of pooled urine were loaded to the pre-conditioned column, followed by washing with 10 mL of 10 mM ammonium acetate buffer solution. The elution was achieved by using 4 x 5 mL 0.1% formic acid in ACN:methanol (3:1, v/v). The eluent was dried down under the nitrogen stream at room temperature. The residue was reconstituted in initial mobile phase for HPLC-MS-radiodetection analysis. The overall extraction recovery was about 88%.

Pooled fecal samples were processed using multiple solvent extractions with ACN:methanol (3:1, v/v) using 1:3 sample/solvent ratio, followed by centrifugation at 4000 rpm (3220 x g) for 20 min at 4°C. The repeated extraction was stopped when either 80% of the radioactivity had been
recovered or until less than 2% of the radioactivity was extracted. Aliquots of extracted samples were subjected to LSC counting for total radioactivity. The extract was dried down under the nitrogen stream at room temperature, and the final residues were reconstituted in initial mobile phase for LC-MS-radiodetection analysis. An aliquot of the reconstituted solution was subject to LSC radiocounting for extraction recovery calculation. The overall extraction recovery for fecal sample was about 92%.

Method for Metabolite Profiles and Identification

HPLC separation of paritaprevir and metabolites was conducted using a Thermo Accela UHPLC system (Thermo Fisher, San Jose, CA), consisted of an UHPLC autosampler, 1250 Series binary pump and Accela PDA detector. Separation was achieved at room temperature on a Phenomenex Synergy Polar-RP 4 µm, 80 A, 4.6 x 150 mm HPLC column. The HPLC mobile phase consisted of 10 mM ammonium formate (adjusted to pH 3.1 with formic acid) (solvent A) and 100% acetonitrile (solvent B), and the flow rate was maintained at 1.0 mL/min. The gradient was as follows: 0-2 min: 20% B; 2-60 min: 20% - 73% B; 60-63 min: 73% - 95% B; 63 – 63.5 min: 95 % - 20% B; 63.5 - 68min: 20% B. The UHPLC system was interfaced with a Thermo Fisher Orbitrap Discovery™ mass spectrometer (Thermo Fisher Scientific). The mass spectrometric analyses were conducted using electrospray ionization (ESI) operated in positive ionization mode. The MS settings were as follows: ESI voltage: 4.0 kV, capillary temperature 300 °C, capillary voltage 2 V and tube lens 105 V. The sheath gas was set to 35.0 arbitrary units and auxiliary gas to 10 arbitrary units. The unchanged parent drug and its metabolites were detected using data dependent multiple-stage mass analysis with mass isolation of 2 Da, normalized collision energy of 35% for both MS² and MS³. The mass resolution was set for 30000 for full scan and 7500 for MS² and MS³ scans. Accurate mass measurement was obtained.
using daily external calibration. Data acquisition and processing were carried out using Xcalibur 2.2 (Thermo Fisher Scientific).

Radiolabeled components in plasma, urine or feces samples were detected by Perkin-Elmer TopCount 96 Deep Well Luma Plate (Perkin Elmer, Waltham, MA). The HPLC eluent was split postcolumn between the mass spectrometer and Agilent 1100 fraction collector at a ratio of 20:80. The Agilent 1100 fraction collector was set at 0.3 min interval per well collection. Radioactivity counting was conducted using Perkin-Elmer TopCount NXT system.

Quantitation of Metabolite M2 in Human Plasma

A single liquid-liquid extraction with a mixture of ethyl acetate and hexane was used to extract the compounds of interest from plasma. An aliquot of plasma (200 µL, sample or spiked standard) was combined with 50 µL of internal standard (IS) ((1S,3S,4S)-1-benzyl-4-[(S)-2-[3-cyclopropyl-3-(2-isopropyl-thiazol-4-ylmethyl)-ureido]-3-methyl-butyrylamino]-3-hydroxy-5-phenyl-pentyl)-carbamic acid thiazol-5-ylmethyl ester) in ethanol:water mixture (50:50, v/v)). Following a brief mixing, 850 µL of ethyl acetate and hexane (9:1, by volume) was added to the sample. After mixing and centrifugation, 500 µL of the organic extract was transferred to a clean 96-well plate and evaporated to dryness under a stream of dry nitrogen at room temperature. The samples were reconstituted with 300 µL mobile phase for LC-MS analysis.

M2 and IS were separated on a 50 x 3 mm 2.7 µm Ascentis Express C18 (Supelco) column with an acetonitrile: 0.1% aqueous formic acid mobile phase containing 0.025% trifluoroacetic acid (~60:40, by volume) at a flow rate of 0.6 ml/min. Analysis was performed on a Sciex API-3000 triple quadrupole Mass Spectrometer with a turbo-ionspray interface. Analytes were ionized in the positive ion mode with a source temperature of approximately 450°C. Detection was in the
multiple reaction monitoring (MRM) mode at \( m/z \) 782.3 \( \rightarrow \) 587.2 for M2 and \( m/z \) 747.3 \( \rightarrow \) 322 for IS. M2 and IS peak areas were determined using Sciex TurboQuan software. The plasma drug concentration of each sample was calculated by least squares linear regression analysis of the peak area ratio (parent / IS) of the spiked plasma standards versus concentration.

Pharmacokinetic Calculations

Plasma concentration-time radioactivity data were analyzed with SAS software (version 9.2; SAS Institute Inc., Cary, NC). Maximum plasma concentration (\( C_{\text{max}} \)), time at which \( C_{\text{max}} \) was achieved (\( T_{\text{max}} \)), area under the concentration time curve from time zero to last measurable time point (\( \text{AUC}_{0-t} \)) for total radioactivity, \([^{14}\text{C}]\text{paritaprevir} \), ritonavir and its metabolites in plasma were estimated. Area under the concentration time curve from time zero to infinity (\( \text{AUC}_{0-\infty} \)) and half-life (\( t_{1/2} \)) for total radioactivity, \([^{14}\text{C}]\text{paritaprevir} \) in plasma were also calculated.

In Vitro Studies

**Human Fecal Incubations.** Fresh fecal samples were collected from two healthy male subjects. Aliquots of specimen (approx. 1 gram) were placed into pre-weighted 15 mL centrifuge tubes with screw caps. The sample tubes were kept in an AnaeroPouch (Mitsubishi Gas Chemical Company, Inc.) (Delaney and Onderdonk, 1997; Van Horn et al., 1997) with a PouchAnaero anaerobic gas generating system and an RT AnaeroIndicator before transferring to a CO2 filled Aldrich AtmosBag (glove bag) that was connected to a CO2 cylinder to create an anaerobic environment. An RT AnaeroIndicator was placed inside the bag to monitor the anaerobic conditions. Dulbecco's phosphate buffered saline (DPBS) was degassed by bubbling nitrogen gas through the solution overnight. The fecal samples were diluted with the DPBS to a concentration of \(~100\) mg/mL under a CO2 atmosphere. The samples were mixed by vortexing.
to break up solid matter. An aliquot of fecal homogenate (1.5 mL) was added to the 2 mL Corning cryogenic vials containing [\textsuperscript{14}C]paritaprevir and ritonavir at the final concentrations of 20 µM paritaprevir and 10 µM ritonavir. The vials were capped, vortexed and placed in an AnaeroPouch with a PouchAnaero anaerobic gas generating system and an RT AnaeroIndicator. The pouches were sealed before removing from the glove bag. The pouches containing sample vials were placed in a 37°C water bath for 6, 24 and 48 hr. Anaerobic conditions were maintained throughout the study as monitored by the RT AnaeroIndicator. At the end of the incubation, sample was mixed with 1.5 mL acetonitrile/methanol (3:1, v/v). After centrifugation, aliquots of supernatants were injected for HLPC-radioflow analysis or HPLC-MS-radiodetection analysis.

**Incubations in Human Hepatocytes.** The cryo-preserved hepatocytes (10 donor pool) were obtained from Celsis *In Vitro* Technologies (Baltimore, MD, lot# VRR). The hepatocyte suspension was diluted to 500,000 viable cells/mL. The incubations were conducted with or without chemical inhibitors. For the incubations with inhibitors, either ritonavir or 1-aminobenzotriazole was added to 0.5 mL of the diluted hepatocyte suspensions, at a final concentration of 1 µM or 1 mM for ritonavir or 1-aminobenzotriazole, respectively. The plates were incubated for 30 minutes at 37°C. [\textsuperscript{14}C]paritaprevir was then added to the pretreated hepatocyte suspension solution, at a final concentration of 1 µM, incubated for 4 and 24 hr at 37°C. At the end of incubation, 1 mL mixture of acetonitrile/ethanol (50/50, v/v) was added to quench the reaction. Solutions were mixed thoroughly, spun down and stored at –20°C until analysis.

**Incubations in Simulated Intestinal Fluids.** The simulated intestinal fluids (SIF) were prepared by dissolving SIF power (Biorelevant, Croydon Surrey, UK) into phosphate buffer
solution (pH 6.52) for fasted state simulated intestinal fluid (FaSSIF), or into phosphate buffer solution (pH 5.00) for fed state simulated intestinal fluid (FeSSIF). [14C]Paritaprevir was added to FaSSIF or FeSSIF solutions to the final concentration of 10 µM. The solutions were incubated for 4, 8 and 24 hr at 37°C; aliquots of the solutions were subjected to HPLC-radioflow analysis.

Results

Excretion of Radioactivity

Following a single oral dose of [14C]paritaprevir (200 mg and 100 µCi, with 100 mg ritonavir) to four healthy volunteers, the excretion of radioactivity in urine and feces from all the subjects was measured up to 192 hours post dose. The mean cumulative percent of radioactive dose recovered in urine and feces is illustrated in Fig. 2. The overall mean recovery of radioactivity in urine and feces samples was 96.5% over the 192-hour study, with recovery in individual subjects ranging from 96.0 to 96.9%. Most of the administered radioactivity was recovered in the first 120 hours post dose (90.5%). The radioactivity was excreted primarily through fecal elimination (mean, 87.8% of dose). Renal excretion was relatively minor (mean, 8.76% of dose).

Pharmacokinetic Data Analysis

The pharmacokinetic parameters for paritaprevir, ritonavir and total radioactivity are summarized in Table 1. The concentration of total radioactivity was measured by LSC, expressed as ng-equivalent/g. The concentrations of paritaprevir and ritonavir were determined using a validated LC-MS/MS bioanalytical method, expressed as ng/mL. Paritaprevir and total radioactivity T_max occurred approximately 3-4 hours following single oral dose. Mean peak plasma concentration (C_max) for the paritaprevir and total radioactivity were 886 ng/mL and 1155
ng-eq/g, respectively. The concentrations of paritaprevir and total radioactivity apparently declined in parallel after reaching the peak concentration (Fig. 3). The AUC$_{0\text{--}last}$ for the parent drug and total radioactivity were 4610 ± 3440 ng·h/mL and 4960±3600 ng-eq·h/g, respectively. The AUC$_{0\text{--}last}$ of paritaprevir measured by LC-MS bioanalytical method represents greater than 90% of total plasma radioactivity AUC, suggesting that paritaprevir is the primary component in circulation.

Metabolite Profiles of [14C]paritaprevir in Excreta and Circulation

**Plasma.** Representative HPLC radiochromatograms of [14C]paritaprevir and its metabolites in pooled human plasma are shown in Fig. 4. [14C]Paritaprevir is the predominant component in human plasma, metabolite M2 and M29 are present as minor component. Low levels of M13 were also detected but only in the 2 hr pooled sample. In addition, trace level metabolites M3 and M6 are detected only by LC-MS analysis. The average percent of plasma radioactivity for [14C]paritaprevir and its metabolites in time-point weighted AUC$_{0\text{--}12h}$ pooled plasma (Hamilton pooling) is tabulated in Table 2. Unchanged parent drug represented average 90.1% of radioactivity in the pooled plasma and metabolite M2 accounted for ~6.5% of drug-related material in plasma. Other metabolites include M29 (2.1% of total radioactivity in plasma) and M3 (1.2% of total radioactivity in plasma).

**Urine and feces.** Chromatographic evaluation of urine samples showed similar metabolites identified in human plasma, including paritaprevir, M2, M13 and M29. After 72 hr post dose to the last collection time point 144-168 hr, M13 was the only radiochemical component detected in urine. The representative HPLC radiochromatogram of pooled human urine is shown in Fig. 5A). M13 was the most significant component in urine throughout entire sample collection period,
accounting for 8.6% of dose; unchanged parent drug and other metabolites were present at low to trace levels with respect to the administered dose. The mean quantification results for paritaprevir and metabolites in urine and feces, expressed as the percentages of the administered radioactive dose, are tabulated in Table 3.

The representative HPLC radiochromatogram of pooled human feces is shown in Fig.5B. Paritaprevir was extensively degraded in feces. Unchanged parent drug only accounted for 1.1% of total dose, while M29 was the most abundant radiochemical component in feces, accounting for 59.9% of total dose, followed by M2 (8.6%) and co-eluting metabolites M3/M18 (7.5%). Other minor radiochemical components in feces included M24, M22, M23, M13, M14, M17, and M6 (each ≤ 3.3% of dose) (Table 3). The proposed metabolic scheme for paritaprevir in humans is shown in Fig. 6.

**LC-MS/MS Characterization of the Metabolites**

As described under Method for Metabolite Profiles and Identification, metabolites of paritaprevir were characterized using a combination of positive ionization high resolution full scan MS and product ion scan (MS/MS) analyses. The structures of the metabolite M2 and M13 were confirmed against the reference materials and the structures of other metabolites were proposed based on the high resolution MS/MS fragmentation pattern analysis. The approximate retention time and key mass spectral fragmentation of paritaprevir and metabolites are listed in Table 4.

**Paritaprevir.** Parent drug yielded a protonated molecular ion ([M+H]^+) at m/z 766.3003 (calculated mass m/z 766.3017, C_{40}H_{44}N_{7}O_{7}S^+) in positive ion mode. The key fragments of m/z 766 is listed in Table 4. The assignment of key MS/MS fragment ions at m/z 571.2331 (base peak) (loss of phenanthridin-6-ol), m/z 553.2228 (loss of phenanthridin-6-ol and H_{2}O), m/z 543.2383
(loss of phenanthridin-6-ol and CO), \( m/z \) 450.2132 (loss of phenanthridin-6-ol and
cyclopropanesulfonamide) and \( m/z \) 422.2187 (predicted molecular formula as \( \text{C}_{23}\text{H}_{28}\text{N}_{5}\text{O}_{3}^+ \), a
loss of CO from product ion at \( m/z \) 450). The CID spectrum and fragmentation pathway of
paritaprevir was provided in supplemental materials.

**Metabolite M2.** M2 produced a protonated molecular ion at \( m/z \) 782.2949, which is 16 amu
higher than that of parent drug and consistent with the chemical formula \( \text{C}_{40}\text{H}_{44}\text{N}_{7}\text{O}_{8}^+ \)
(calculated mass \( m/z \) 782.2967, parent + O). The CID of M2 (\( m/z \) 782) produced major fragment
ions including the base peak at \( m/z \) 587.2291 (loss of phenanthridin-6-ol), \( m/z \) 559.2337 (loss of
phenanthridin-6-ol and CO), \( m/z \) 502.1757 (loss of pyrrole and \( \text{H}_2\text{O} \) from fragment ion \( m/z \) 587),
\( m/z \) 474.1808 (-CO from \( m/z \) 502), and \( m/z \) 422.1747 (predicted formula as \( \text{C}_{20}\text{H}_{28}\text{N}_{3}\text{O}_{5}^+ \), loss
of methylpyrazine-2-carboxamide from fragment ion \( m/z \) 559). Note that fragment ions as a
result of amide cleavage to loss cyclopropanesulfonamide were not observed in either MS/MS or
MS\(^3\) experiment. Metabolite M2 was confirmed by using the synthetic reference material by
comparison of MS/MS pattern and co-injection LC-MS analysis. Metabolite M2 generated from
HLM incubations has also been isolated and characterized by NMR spectroscopy (data shown in
supplemental materials), which is consistent with the NMR spectra of M2 reference material to
support the structure assignment.

**Metabolite M3.** M3, present at trace level in plasma, gave a protonated molecular ion of \( m/z \)
798.2900, indicating addition of two oxygen atoms to the parent drug (predicted formula
\( \text{C}_{40}\text{H}_{44}\text{N}_{7}\text{O}_{9}^+ \), calculated accurate mass \( m/z \) 798.2916). The major fragment ions were \( m/z \)
603.2240 (loss of phenanthridin-6-ol), \( m/z \) 575.2292 (loss of phenanthridin-6-ol and CO), \( m/z \)
518.1710 (loss of pyrrole and \( \text{H}_2\text{O} \) from fragment ion \( m/z \) 603) and \( m/z \) 422.1748 (predicted
formula as \( \text{C}_{20}\text{H}_{28}\text{N}_{3}\text{O}_{5}\text{S}^+ \). M3 is tentatively assigned as a hydroxylated M2, where the hydroxylation likely occurs at the methylpyrazine moiety.

**Metabolite M6.** M6, present at trace level in plasma, gave a protonated molecular ion of \( m/z \) 782.2950, indicating addition of one oxygen atom to the parent drug (predicted formula \( \text{C}_{40}\text{H}_{44}\text{N}_{7}\text{O}_{8}\text{S}^+ \)). The major fragment ions of M6 included \( m/z \) 587.2281 (loss of phenanthridin-6-ol), \( m/z \) 569.2170 (loss of phenanthridin-6-ol and \( \text{H}_2\text{O} \)), \( m/z \) 559.2327 (loss of phenanthridin-6-ol and \( \text{CO} \)). MS\(^3\) of \( m/z \) 587 produced major fragment ion at \( m/z \) 569.2170 (-\( \text{H}_2\text{O} \)) and 474.1804 (loss of pyrrolidine-2-carbaldehyde from \( m/z \) 569). Therefore, M6 was assigned as a hydroxylated metabolite and hydroxylation possibly occurred at the hydrocarbon region in the macrocyclic ring.

**Metabolite M13.** A polar metabolite M13 was observed at solvent front in LC-MS, with a protonated ion of \( m/z \) 139.0497, suggesting a molecular formula of \( \text{C}_6\text{H}_7\text{N}_2\text{O}_2^+ \) (calculated exact mass: 139.0502). The MS2 analysis of M13 showed the major fragment at \( m/z \) 121.0392 (a loss of water). M13 was assigned as 5-methylpyrazine-2-carboxylic acid, based accurate mass and similar fragmentation compared to that of 5-methylpyrazine 2-carboxylic acid standard.

**Metabolite M29.** M29 gave a protonated molecular ion of \( m/z \) 663.2916, suggesting a molecular formula of \( \text{C}_{37}\text{H}_{39}\text{N}_6\text{O}_6^+ \). The major fragments of M29 included \( m/z \) 468.2246 (loss of phenanthridin-6-ol), 450.2133 (loss of phenanthridin-6-ol and \( \text{H}_2\text{O} \)), 440.2295 (loss of phenanthridin-6-ol and \( \text{CO} \)), 422.2185 (loss of phenanthridin-6-ol and carboxylic acid). The accurate mass data suggested the loss of cyclopropanesulfonamide from parent drug. Therefore, M29 was assigned as the amide hydrolytic product of parent drug with a loss of the sulfonamide.
Metabolite M14, M16, M18, M19, M21. M14, M16, M18, M19 and M21 were only detected in feces at low levels; most of these were below radiochemical detection limit. These metabolites gave protonated molecular ion at \( m/z \) 679.2863, which is consistent with the predicted formula of \( \text{C}_{37}\text{H}_{39}\text{N}_6\text{O}_7^+ \) (calculated mass 679.2875). CID spectra of M14 and M19 showed the presence of fragment ion at \( m/z \) 468.2240 (loss of phenanthridine-diol), indicating hydroxylation occurred at phenanthridine moiety. The hydroxylation position for M16, M18 and M21 was not further characterized due to the fact that overall abundance of these metabolites is low in feces.

Metabolite M15, M17, M20. M15, M17 and M20 were also exclusively detected in feces at trace to low levels. For M15, M17 and M20, all gave protonated molecular ion at \( m/z \) 798.2894, which is consistent with the predicted formula of \( \text{C}_{40}\text{H}_{44}\text{N}_7\text{O}_9\text{S}^+ \), indicating addition of two oxygen atoms to the parent drug. CID of M15 produced key fragment ions at \( m/z \) 603.2228 (loss of phenanthridin-6-ol) and 585.2125 (loss of H\(_2\)O from ion \( m/z \) 603). The presence of fragment ion at \( m/z \) 438.1691 (as a result of loss of phenanthridin-6-ol and methylpyrazine-2-carboxamide) and \( m/z \) 420.1585 (loss from ion \( m/z \) 438) suggests that M15 has the hydroxylation likely at macrocyclic region. M17 gave CID key fragment ions at \( m/z \) 603.2240, 575.2294 (loss of phenanthridin-6-ol and CO) and 422.1750. M17 is tentatively assigned as a di-hydroxylate metabolite. M20 produced CID key fragment ions at 587.2277 (loss of phenanthridine-diol), 559.2326 (loss of phenanthridine-diol and H\(_2\)O) and 422.1741. M20 is tentatively as hydroxylate metabolite of M2 with hydroxylation at phenanthridine moiety.

Metabolite M22, M23. Metabolite M22 and M23 were only detected in feces; both gave the protonated molecular ion at \( m/z \) 697.2965, which is consistent with the predicted formula of \( \text{C}_{37}\text{H}_{41}\text{N}_6\text{O}_8^+ \) (calculated mass 697.2980), indicating addition of one oxygen atom and one water
to M29. The presence of CID fragment ion at \( m/z \) 468.2230 (loss of C\(_{13}\)H\(_{11}\)NO\(_3\) ) from M23 suggests that oxidation occurs at the phenanthridine moiety. The structure of M22 was not elucidated due to ion intensity and poor quality of CID spectrum.

**Metabolite M24.** Metabolite M24 was only observed in feces; it gave the protonated molecular ion at \( m/z \) 903.3138, which is consistent with the chemical formula C\(_{43}\)H\(_{51}\)N\(_8\)O\(_{10}\)S\(_2\)\(^+\) (calculated mass 903.3164), indicating possible addition of one oxygen atom and one cysteine to the parent drug. The major fragment ions were 885.3042 (-H\(_2\)O), 782.2951 (-Cysteine), 764.2854 (loss of H\(_2\)O and Cysteine), 690.2369 (loss of phenanthridin-6-ol and H\(_2\)O). The presence of fragment ion at \( m/z \) 690.2369 indicates that oxidation and cysteine conjugation occurs at the macrocyclic olefinic linker.

**Metabolite M25.** Metabolite M25 was only observed in feces; it gave the protonated molecular ion at \( m/z \) 646.2683, which is consistent with the predicted formula of C\(_{34}\)H\(_{40}\)N\(_5\)O\(_6\)S\(^+\) (calculated mass 646.2694). M25 is proposed to be an amide hydrolysis metabolite from parent drug with a loss of methylpyrazine-2-carboxylic acid.

**Metabolite M26.** Metabolite M26 was only observed in feces; it gave the protonated molecular ion at \( m/z \) 543.2591, which is consistent with the predicted formula of C\(_{31}\)H\(_{35}\)N\(_4\)O\(_5\)\(^+\) (calculated mass 543.2602). M26 is proposed to be an amide hydrolysis metabolite from M29 with a loss of methylpyrazine-2-carboxylic acid.

**Metabolite M2 in Human Plasma at Steady State**

As discussed previous section, metabolite M2 is the main metabolite in circulation, representing approximately 6.5% following a single oral dose of \(^{14}\)Cparitaprevir/ritonavir. The plasma concentration of metabolite M2 at the steady state was also determined following multiple oral
dosing with paritaprevir/ritonavir in humans. Eight subjects received a 300 mg dose of paritaprevir, in combination with a 100 mg dose of ritonavir once daily for 14 consecutive days. Paritaprevir peak plasma concentrations averaged 6398 ng/ml (Table 5). M2 peak plasma concentration was substantially lower, with a mean concentration of 21.7 ng/ml. Paritaprevir AUC values averaged 33595 ng•hr/ml; M2 AUC values followed the trend noted in the C_max values, with a mean value of 208.5 ng•hr/ml. The M2 AUC values as a percentage of the A-1043422 AUC averaged 0.6%.

Metabolite formation in *in vitro* fecal incubation

Biotransformation of paritaprevir by human intestinal microflora was evaluated by incubations of [14C]paritaprevir (20 μM) with ritonavir (10 μM) in freshly prepared human fecal homogenate under anaerobic conditions. Fig. 7 shows representative radiochromatograms of [14C]paritaprevir in human fecal homogenate incubations, indicating that paritaprevir rapidly undergoes extensive hydrolysis to form M29. M29 represented approximately 80% of the total radioactivity after a 6 hr incubation, and approximately 95% after a 24 hr incubation. In addition, the hydrolysis products M13 (representing 1-3% of the total radioactivity), and M26 (an amide hydrolysis product from M29 with a loss of M13) were also observed by LC-MS analysis. The results confirm that unchanged paritaprevir from biliary elimination and unabsorbed paritaprevir can readily be hydrolyzed to M29 and M13 in human *in vivo*.

*In vitro* hydrolysis in simulated intestinal fluids

The stability of paritaprevir in simulated intestinal fluids was evaluated by incubating [14C]paritaprevir at 10 μM in either fasted state simulated intestinal fluid (FaSSIF, pH6.52) or fed state simulated intestinal fluid (FeSSIF, pH 5.0) solutions for 8 and 24 hr. HPLC
radiochromatographic analysis indicated that paritaprevir was stable in both FaSSIF and FeSSIF solutions; no hydrolysis products were detected, indicating sulfonamide or amide hydrolysis is unlikely to occur chemically in human intestinal fluids either under fast or fed conditions.

**Metabolite formation in *in vitro* human hepatocyte incubation**

The *in vitro* metabolite profile in human hepatocytes was evaluated by incubations of [14C]paritaprevir with or without cytochrome P450 inhibitors (ritonavir or 1-aminobenzotriazole). As shown in Fig. 8 (a & b), [14C]paritaprevir generates oxidative metabolites M2 and M3 (confirmed by LC-MS) in human hepatocytes in absence of chemical inhibitors. When either ritonavir or pan CYP inhibitor 1-aminobenzotriazole was co-incubated with paritaprevir, [14C]paritaprevir is the only radioactive component detected, and formation of oxidative metabolites (M2 and M3) was completely inhibited by these chemical inhibitors (Fig. 8 (c) co-incubation with ritonavir and (d) co-incubation with 1-aminobenzotriazole). Note that hydrolysis metabolites (e.g. M29 and M13) were not detected in human hepatocytes with or without chemical inhibitors, suggesting that hydrolysis products M29 and M13 are not formed by hepatic enzymes.

**Discussion**

The mass balance, disposition and metabolism of paritaprevir were evaluated in four healthy human subjects. Following administration of a single 200-mg oral dose of [14C] paritaprevir, co-administered with a single 100-mg oral dose of ritonavir, the mean total recovery of administered radioactive dose was 96.5%, with recovery in individual subjects ranging from 96.0 to 96.9%. The majority of the administered radioactive dose (87.8%) was excreted in feces. Renal elimination plays a minor role, since radioactivity recovered in urine only accounted for 8.8% of
dose, indicating that paritaprevir and metabolites are primarily eliminated in humans through hepatobiliary and fecal elimination routes.

Metabolites of paritaprevir in plasma, urine and feces were profiled using HPLC-radioactivity detection and structures of metabolites were characterized using HPLC-high resolution mass spectrometry. Biotransformation of paritaprevir in humans involves: 1) CYP-mediated oxidation on the olefinic linker, the phenanthridine group, the methylpyrazinyl group, or combinations thereof; 2) amide hydrolysis at the acyl cyclopropane-sulfonamide moiety and the pyrazine-2-carboxyamide moiety. The CYP-mediated oxidation of paritaprevir is greatly attenuated with ritonavir co-dosing via inhibition of CYP3A-mediated first pass metabolism.

Of the total radioactivity excreted in feces, the acyl sulfonamide hydrolysis product M29 was the primary radiochemical component (~60% of dose), followed by the oxidative metabolite M2 (8.6% of dose). Paritaprevir recovered in feces and urine represented 1.2% of dose. Amide hydrolysis product M13 was the primary component found in human urine, accounting for 8.6% of dose. In vitro incubations of [14C]paritaprevir in human fecal homogenate showed that paritaprevir is extensively hydrolyzed to form primarily M29 (>80% of radioactivity in a 6 hr incubation), and M13 to a much less extent (~1-3% of radioactivity) and M26, indicating the important role of human intestinal micro flora in amide hydrolysis of paritaprevir. Fecal degradation of paritaprevir has also been observed in preclinical toxicology species (unpublished data). In vitro stability assessment of [14C]paritaprevir in simulated intestinal fluids indicated that [14C]paritaprevir is chemically stable in human intestinal fluids under either fasted or fed conditions. In addition, the absence of hydrolysis products (M29 and M13) in in vitro human hepatocyte assays with or without chemical inhibitors (ritonavir or 1-aminobenzotriazole)
suggests that the formation of hydrolysis products M29 and M13 is not mediated by hepatic enzymes.

Following a single dose of [14C]paritaprevir with ritonavir, [14C]paritaprevir was the major component in plasma, and five minor metabolites M2, M3, M6, M13 and M29 were identified. Unchanged parent drug represented an average of 90.1% of radioactivity in plasma, and metabolite M2 accounted for ~6.5% of drug-related material in plasma. Other metabolites include M29 and M3 (2.1 and 1.2% of total radioactivity in plasma, respectively). Steady state concentrations of M2 in plasma samples obtained from subjects receiving multiple doses of 300/100 mg paritaprevir/ritonavir further confirm that there are no major circulating metabolites in human plasma. M2 is a nonreactive stable metabolite likely formed presumably through the epoxide intermediate. Since the overall metabolism contribution to the elimination of paritaprevir is minimal, the safety risk associated with the M2 metabolite and its formation pathway is remote. Clinically concomitant administration of strong CYP3A4 inhibitors or inducers may cause a modest increase or decrease in paritaprevir and ritonavir systemic exposures. Detailed discussion of potential drug-drug interaction mechanisms involving paritaprevir/ritonavir and other components in the DAA regimen is reported elsewhere (Shebly et al. 2016).

In summary, the overall disposition and metabolism of 200 mg [14C]paritaprevir co-dosed with 100-mg ritonavir in healthy volunteers was investigated. The overall study objectives were met, with good recovery of radioactivity dose from all subjects. The mass balance results confirm that orally administered paritaprevir is primarily eliminated by the biliary-fecal route. Paritaprevir is extensively degraded through microflora mediated hydrolysis. The structures of metabolites in circulation and excreta were elucidated, with proposed metabolic pathways of
CYP3A4 mediated oxidation which is greatly inhibited by co-administered ritonavir and microflora-mediated hydrolysis.
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Authorship Contributions

Participated in research design: Shen, Menon, Kavetskaia, Fischer.

Conducted experiments: Serby, Zhang, Marsh.

Contributed new reagents or analytic tools: Serby, Reed.

Performed data analysis: Shen, Serby, Marsh, Khatri, Menon.

Wrote or contributed to the writing of the manuscript: Shen, Lee, Kavetskaia, Fischer, Serby, Marsh, Khatri, Menon, Reed, Zhang.
References


Unnumbered Footnote to the Title

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Figures

Fig. 1. Structure of paritaprevir. Asterisk denotes position of $[^{14}\text{C}]$ radiolabel.

Fig. 2. Mean cumulative percent of radioactive dose recovered in urine and feces at specified intervals after a single 200-mg oral dose of $[^{14}\text{C}]$ paritaprevir, co-administered with single 100-mg oral dose of ritonavir to healthy male subjects.

Fig. 3. Mean (standard deviation) plasma concentration-time curves for paritaprevir (ng/mL) and total radioactivity (ng-eq/g) in male subjects administered a single 200-mg oral dose of $[^{14}\text{C}]$ paritaprevir, co-administered with single 100-mg oral dose of ritonavir (n=4).

Fig. 4. Representative HPLC radiochromatograms of paritaprevir and its metabolites in human plasma after a single 200-mg oral dose of $[^{14}\text{C}]$ paritaprevir, co-administered with single 100-mg oral dose of ritonavir to healthy male subjects.

Fig. 5. Representative HPLC radiochromatograms of paritaprevir and its metabolites in human excreta, (A) urine and (B) feces, after a single 200-mg oral dose of $[^{14}\text{C}]$ paritaprevir, co-administered with a single 100-mg oral dose of ritonavir to healthy male subjects.

Fig. 6. Proposed metabolic pathways of paritaprevir in humans.

Fig. 7. Representative HPLC radiochromatograms of 20 µM $[^{14}\text{C}]$paritaprevir with 10 µM ritonavir following incubations in fresh human fecal homogenate for 0 hr (A) and 6 hr (B).

Fig. 8. Representative HPLC radiochromatograms of 1 µM $[^{14}\text{C}]$paritaprevir following incubations in human hepatocytes for 0 hr (A), 24 hr (B), 24 hr with cytochrome P450 inhibitor ritonavir (C) or 1-aminobenzotriazole (D).
### Tables

Table 1. Pharmacokinetic parameters (mean ± SD) of paritaprevir, ritonavir and total radioactivity in plasma following a 200/100 mg oral dose of [14C]paritaprevir /ritonavir

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$C_{\text{max}}$ (ng-eq/g or ng/mL)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$\text{AUC}_{0-\text{last}}$ (ng-eq•h/g or ng•h/mL)</th>
<th>$\text{AUC}_{0-\infty}$ (ng-eq•hr/g or ng•h/mL)</th>
<th>$T_{\frac{1}{2}}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Radioactivity</td>
<td>1155±798 *</td>
<td>3.5±1.9</td>
<td>4960±3600 *</td>
<td>6952±2297 *</td>
<td></td>
</tr>
<tr>
<td>paritaprevir</td>
<td>886 ± 774</td>
<td>3.0 ± 1.2</td>
<td>4610 ± 3440</td>
<td>4630 ± 3430</td>
<td>5.27 ± 1.08</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>998 ± 569</td>
<td>4.5 ± 1.9</td>
<td>5460 ± 1960</td>
<td>5580 ± 1910</td>
<td>4.01 ± 0.73</td>
</tr>
</tbody>
</table>

* Total radioactivity Cmax unit – ng-eq/g. AUC unit - ng-eq*hr/g.
Table 2. Average percent of radioactivity in time-point weighted AUC(0-12h) pooled human plasma (n=4)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>% Radioactivity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Paritaprevir</td>
<td>90.1  13.0</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>6.5  9.4</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>1.2  2.4</td>
<td></td>
</tr>
<tr>
<td>M29</td>
<td>2.1  2.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Percentages of excretory metabolites of in humans following administration of a single 200/100 mg oral dose of [14C]paritaprevir /ritonavir (n=4)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Feces 0-192 h</th>
<th>Urine 0-168 h</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Radioactivity</td>
<td>87.8</td>
<td>8.76</td>
<td>1.15</td>
</tr>
<tr>
<td>Paritaprevir</td>
<td>1.1</td>
<td>0.05</td>
<td>1.15</td>
</tr>
<tr>
<td>M2</td>
<td>8.55</td>
<td>0.13</td>
<td>8.68</td>
</tr>
<tr>
<td>M6</td>
<td>0.78</td>
<td>ND</td>
<td>0.78</td>
</tr>
<tr>
<td>M3/M18*</td>
<td>7.47</td>
<td>ND</td>
<td>7.47</td>
</tr>
<tr>
<td>M13</td>
<td>1.67</td>
<td>8.57</td>
<td>10.2</td>
</tr>
<tr>
<td>M14</td>
<td>1.12</td>
<td>ND</td>
<td>1.12</td>
</tr>
<tr>
<td>M17</td>
<td>1.08</td>
<td>ND</td>
<td>1.08</td>
</tr>
<tr>
<td>M22/M23**</td>
<td>2.78</td>
<td>ND</td>
<td>2.78</td>
</tr>
<tr>
<td>M24</td>
<td>3.32</td>
<td>ND</td>
<td>3.32</td>
</tr>
<tr>
<td>M29</td>
<td>59.9</td>
<td>0.01</td>
<td>59.9</td>
</tr>
</tbody>
</table>

* M3 and M18 were co-eluting metabolites in fecal sample analysis.
** M22 and M23 were co-eluting metabolites in fecal sample analysis.
ND – not detected
Table 4. Retention time, molecular ion and characteristic fragment ions of paritaprevir and metabolites in human plasma, urine or feces

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approx. RT (min)</th>
<th>Obs. [M+H]+</th>
<th>Calc. [M+H]+</th>
<th>Δppm</th>
<th>Metabolite ID</th>
<th>Key Fragment Ions (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>paritaprevir</td>
<td>50.4</td>
<td>766.3003</td>
<td>766.3017</td>
<td>-1.8</td>
<td>Parent drug</td>
<td>571, 553, 543, 458, 450, 422, 406, 355</td>
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<tr>
<td>M29</td>
<td>39.1</td>
<td>663.2916</td>
<td>663.2926</td>
<td>-1.5</td>
<td>Sulfonamide hydrolysis</td>
<td>468, 450, 440, 422, 303</td>
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<tr>
<td>M2</td>
<td>37.9</td>
<td>782.2949</td>
<td>782.2967</td>
<td>-2.3</td>
<td>Parent drug + O</td>
<td>587, 559, 502, 474, 422, 327</td>
</tr>
<tr>
<td>M6</td>
<td>35.8</td>
<td>782.2951</td>
<td>782.2967</td>
<td>-2.1</td>
<td>Parent drug + O</td>
<td>764, 618, 587, 569, 559, 541, 474</td>
</tr>
<tr>
<td>M3</td>
<td>32.1</td>
<td>798.2900</td>
<td>798.2916</td>
<td>-2.0</td>
<td>Parent drug + 2O</td>
<td>603, 575, 518, 490, 422</td>
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<tr>
<td>M13</td>
<td>~3</td>
<td>139.0498</td>
<td>139.0502</td>
<td>-3.1</td>
<td>5-methylpyrazine-2-carboxylic acid</td>
<td>121, 95</td>
</tr>
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</table>

Feces Specific Metabolites

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approx. RT (min)</th>
<th>Obs. [M+H]+</th>
<th>Calc. [M+H]+</th>
<th>Δppm</th>
<th>Metabolite ID</th>
<th>Key Fragment Ions (m/z)</th>
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<tbody>
<tr>
<td>M14</td>
<td>34.6</td>
<td>679.2863</td>
<td>679.2875</td>
<td>-1.8</td>
<td>M29 + O</td>
<td>468, 440, 422, 373, 355, 303</td>
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<tr>
<td>M15</td>
<td>34.1</td>
<td>798.2894</td>
<td>798.2916</td>
<td>-2.8</td>
<td>Parent drug + 2O</td>
<td>780, 603, 585, 518, 438, 420</td>
</tr>
<tr>
<td>M16</td>
<td>33.1</td>
<td>679.2863</td>
<td>679.2875</td>
<td>-1.8</td>
<td>M29 + O</td>
<td>484, 468, 456, 319</td>
</tr>
<tr>
<td>M17</td>
<td>32.8</td>
<td>798.2894</td>
<td>798.2916</td>
<td>-2.8</td>
<td>Parent drug + 2O</td>
<td>603, 575, 557, 518, 490, 422, 327</td>
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<tr>
<td>M18</td>
<td>32.5</td>
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<td>484, 466, 456, 438, 371, 303</td>
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<tr>
<td>M19</td>
<td>31.8</td>
<td>679.2863</td>
<td>679.2875</td>
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<td>M29 + O</td>
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<td>M20</td>
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<td>798.2894</td>
<td>798.2916</td>
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<td>M21</td>
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<td>-1.8</td>
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<td>661, 617, 484, 466</td>
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<tr>
<td>M22</td>
<td>22.1</td>
<td>697.2965</td>
<td>697.2975</td>
<td>-1.4</td>
<td>M29 + O +H2O</td>
<td>accurate mass only</td>
</tr>
<tr>
<td>M23</td>
<td>21.7</td>
<td>697.2965</td>
<td>697.2975</td>
<td>-1.4</td>
<td>M29 + O +H2O</td>
<td>679, 468, 450, 440, 303</td>
</tr>
<tr>
<td>M24</td>
<td>18.9</td>
<td>903.3148</td>
<td>903.3164</td>
<td>-1.8</td>
<td>Parent+O+Cysteine</td>
<td>885, 867, 782, 764, 690, 569, 541, 457, 422</td>
</tr>
<tr>
<td>M25</td>
<td>36.1</td>
<td>646.2683</td>
<td>646.2694</td>
<td>-1.7</td>
<td>Amide hydrolysis</td>
<td>525, 497, 451, 423, 406, 345, 330, 302</td>
</tr>
<tr>
<td>M26</td>
<td>23.9</td>
<td>543.2591</td>
<td>543.2602</td>
<td>-2.0</td>
<td>Amide hydrolysis of M29</td>
<td>348, 320, 304, 302, 287</td>
</tr>
</tbody>
</table>

# Observed only in fecal samples.
Table 5. Pharmacokinetics of paritaprevir and metabolite M2 after multiple oral dosing with paritaprevir/ritonavir in Man (Day 14)

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hr)</th>
<th>AUC&lt;sub&gt;0-24&lt;/sub&gt; (ng•hr/ml)</th>
<th>AUC % *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paritaprevir</td>
<td>6398 (4036)</td>
<td>3</td>
<td>33595 (19380)</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>21.7 (17.3)</td>
<td>5</td>
<td>208.5 (138.5)</td>
<td>0.6 (0.3)</td>
</tr>
</tbody>
</table>

* AUC% (metabolite AUC as a percentage of parent drug
Fig 1
Fig. 2

Cumulative excretion (% of dose)

Hours post-dose (h)

- Urine
- Feces
- Total radioactivity
Fig. 3

[Graph showing plasma concentration of $[^{14}C]$paritaprevir over hours post-dose. The x-axis represents hours post-dose (hr) from 0 to 48, and the y-axis represents plasma concentration (ng/mL or ng-eq/g) on a logarithmic scale from $10^0$ to $10^4$. Two lines are shown: one for total radioactivity and one for paritaprevir.]
Fig. 4

Pooled plasma at 2 hr

Pooled plasma at 12 hr

CPM

Time (min)
Fig. 5

A) Pooled urine 0-12h

B) Pooled feces 24-48h
Fig. 7

A) 0 hr

B) 6 hr

paritaprevir

impurity

M29

M13

impurity
Fig. 8

A) paritaprevir

B) paritaprevir

C) paritaprevir

D) paritaprevir