Characterization of Pharmacokinetics, Biotransformation and Elimination of Pomotrelvir Orally Administered in Healthy Male Adults Using Two $^{14}$C-labeled Microtracers with Separate Labeling Positions

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List of nonstandard abbreviations:

Pomotrelvir (7-chloro-N-((S)-1-(((S)-1-cyano-2-((S)-2-oxopiperidin-3-yl)ethyl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-1H-indole-2-carboxamide); AME, absorption, metabolism, and excretion; AMS, accelerator mass spectrometry; AUC, area under the plasma concentration-time curve; CoVs, coronaviruses; COVID-19, Coronavirus Disease 2019; CL, clearance; CV, coefficient of variation; DAA, direct-acting antiviral; DDI, drug-drug interaction; EUA, US regulatory emergency use authorization; HPLC, high performance liquid chromatography; LC, liquid chromatography; LSC, liquid scintillation counting; Mpro, main protease; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PK, pharmacokinetics; SARS-CoV-2, severe acute respiratory syndrome CoV-2; t1/2, half-life; T_{max}, time of maximum observed plasma concentration.
Abstract

Pomotrelvir is an orally bioavailable, target antiviral inhibitor of the main protease (Mpro) of coronaviruses (CoVs), including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiological agent of Coronavirus Disease 2019 (COVID-19). The pharmacokinetics, metabolism and elimination of two [14C]-labeled microtracers of 5 µCi/700 mg pomotrelvir with separate labeling positions (isotopomers), [lactam carbonyl-14C-pomotrelvir] and [benzene ring-U-14C-pomotrelvir], following a single oral dose in healthy adult males was evaluated in two separate cohorts. Pomotrelvir was rapidly absorbed and eliminated primarily through metabolism and subsequently excreted via urine and feces. There were no differences in pomotrelvir pharmacokinetics between the two cohorts. The mean total radioactive dose recovered was 93.8% (n=8) in the lactam cohort (58% in urine and 36% in feces) and 94.2% (n=8) in the benzene cohort (75% in urine and 19% in feces), with ≥ 80% of [14C] recovered within 96 hours after dosing. About 5% and 3% of the intact pomotrelvir was recovered in feces and urine, respectively. Eleven major metabolites were detected and characterized using LC-accelerator mass spectrometry (AMS) and LC-MS/MS methods, with 3 and 6 different metabolites elucidated in the samples collected from lactam and benzene cohorts, respectively, and 2 metabolites observed in both cohorts. The major metabolism pathway of pomotrelvir is through hydrolysis of its peptide bonds followed by phase II conjugations. These results support that the application of two radiolabeled isotopomers provided a comprehensive metabolite profiling analysis and was a successful approach in identifying the major disposition pathways of pomotrelvir that has complex routes of metabolism.
Significance Statement

An unconventional approach using two differentially labeled $[^{14}C]$ microtracers, [lactam carbonyl-$^{14}$C-pomotrelvir] and [benzene ring-U-$^{14}$C-pomotrelvir] evaluated the mass balance of orally administered pomotrelvir in healthy adult males in two separate cohorts. The radioactive dose recovered in excreta was about 94% for both cohorts. While the two isotopomers of the radiolabeled-pomotrelvir showed no major differences in pharmacokinetics overall, they allowed for differential detection of their radiolabeled metabolites and appropriate characterization of their plasma exposure and excretion in urine and feces.
Introduction

Coronaviruses are known to undergo frequent mutations and genetic recombination (Lau and Chan 2015), which allows the virus to evade natural and vaccine-induced immunity. Despite high vaccination rates, people continue to become infected and reinfected due to the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants (Korber et al. 2020) (Campbell et al. 2021) (Zella et al., 2021). This is due to waning of immunity and mutations of the SARS-CoV-spike protein, which can also cause vaccines to become less effective (Tillett et al., 2021) (Harvey et al., 2021). As there continues to be SARS-CoV-2 infections, additional safe and effective oral agents are needed, especially for older adults and patients with underlying medical conditions at highest risk for progression to serious disease.

Three direct-acting antiviral (DAA) small-molecule protease or polymerase inhibitors (nirmatrelvir, molnupiravir, remdesivir) have received worldwide or US regulatory emergency use authorization (EUA) or approval for treatment of COVID-19 (Nirmatrelvir, Pfizer 2023) (Molnupiravir, Merck & Co 2022) (Remdesivir, Gilead Sciences 2022) and demonstrated reductions in hospitalization and death rates and/or improvement in clinical status in patients with COVID-19, especially for patients who had not been vaccinated or previously infected. However, nirmatrelvir is required to be co-administered with ritonavir and causes significant drug-drug interaction (DDI), while molnupiravir works by inducing viral mutagenesis and has the potential to cause embryo-fetal toxicity. Remdesivir is a polymerase inhibitor, but only available as intravenous administration.

Pomotrelvir is an orally bioavailable small molecule, reversible covalent binding, direct-acting inhibitor of the main protease (Mpro) of coronaviruses (CoVs), including SARS-CoV-2 that causes Coronavirus Disease 2019 (COVID-19). Pomotrelvir has broad-spectrum activity against SARS-CoV-2 variants and CoV Mpro in cell-based antiviral and in vitro enzyme assays, respectively (Tong et al., submitted 2023). Additionally, in vitro resistance studies suggest that pomotrelvir has a high barrier to resistance (Stevens et al., submitted 2023).

When this mass balance study was initiated, two Phase 1 clinical studies in healthy subjects had been completed. These studies revealed that pomotrelvir is well tolerated over a wide (> 10-fold)
of single and multiple ascending oral doses up to 2100 mg/day for 10 days. All study drug-related adverse events (AEs) were mild in severity and resolved. No serious adverse events (SAEs), deaths, or discontinuations due to AEs were reported (Kearney et al., 2022). Pomotrelvir demonstrated dose-linear exposures across doses of 100 to 700 mg when administered with food and demonstrated the ability to achieve and maintain systemic exposures that are anticipated to have potent antiviral activity at the selected clinical dose of 700 mg twice-daily (BID). Evaluation of the DDI potential of pomotrelvir showed that pomotrelvir was a weak inhibitor and not a sensitive substrate of CYP3A4, suggesting no to minimal DDI potential at the proposed clinical dose (Yang et al., 2023).

This study evaluated the pharmacokinetics (PK), metabolism, elimination, and mass balance recovery of a single oral dose of [14C]-pomotrelvir in healthy adult males. Pomotrelvir was anticipated to undergo significant hydrolysis of one of its peptide bonds based on the preliminary metabolite profiling results in animal and clinical studies. In order to track and characterize the major hydrolysis metabolites and their potential secondary metabolites, two radiolabeled-pomotrelvir microtracers with separate labeling positions (isotopomers) were administered in separate cohorts. This is an unconventional approach applied in order to obtain a comprehensive metabolite profile (including identification, relative systematic exposure and excretion pathways) for pomotrelvir, a small molecule with a metabolically labile core structure.
Material and Methods

Radiolabeled Pomotrelvir and Reference Standards

[14C] Pomotrelvir (7-chloro-N-((S)-1-(((S)-1-cyano-2-((S)-2-oxopiperidin-3-yl)ethyl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-1H-indole-2-carboxamide) was separately prepared with a 14C label in the carbonyl carbon atom of the 2-oxopiperidine moiety (lactam) (Figure 1A) and a U-label of the benzene ring of the 7-chloro indole moiety (benzene) (Figure 1B), with specific activity of 57 mCi/mmol and 55 mCi/mmol, respectively by Pharmaron (Cardiff, UK). Unlabeled pomotrelvir as well as metabolite standards were prepared by Pharmaron (Beijing, China). Doses were prepared as an oral solution by combining labeled and unlabeled pomotrelvir to a human dose of 5μCi/700 mg pomotrelvir in polyethylene glycol 400 (PEG 400) and water (97:3, v/v) at a concentration of 20 mg pomotrelvir/mL

Participants

Healthy male adults aged 18–55 years were recruited at a single site (Pharmaron CPC, Inc, Baltimore, MD). To accommodate the objectives of the study, participants were excluded if they had a recent history of incomplete bladder emptying with voiding, or awakening more than once at night to void, or < 1 or > 3 bowel movements per day. Participants who had received 14C-labeled drug within a year or showing 14C content in urine, blood and/or plasma samples significantly exceeding the general environment background at screen were also excluded. All subjects were screened within 28 days before study entry and could only participate in one cohort.

Rationale of Dose Selection

A dose of pomotrelvir 700 mg was chosen for this study as this dose was the clinical dose being studied in the Phase 2 trial and was expected to be high enough to result in measurable pomotrelvir blood levels. The radioactive dose of 5 μCi together with the use of AMS was projected to allow quantification of the radioactivity in blood, plasma, urine and fecal samples for completion of the study objectives, while with minimal radiation exposure risk to healthy participants.
Rationale for Using Two Radiolabeled Isotopomers of Pomotrelvir in the Study

Pomotrelvir was anticipated to undergo hydrolysis by breaking one of the two peptide bonds in vivo, resulting in two major inactive metabolites, PBI-0451A (M306) and S4-Q1 (M167) (Figure 7), which accounted for >20% of the parent drug plasma exposure following oral administration of unlabelled material in preclinical species and humans. With the desire to monitor the systemic exposure and excretion pathways of these hydrolysis metabolites and also their secondary metabolites in this mass balance study, we applied two radiolabeled isotopomers, labelled at two metabolically stable positions, [benzene ring-U-14C]-pomotrelvir and [lactam carbonyl-14C]-pomotrelvir (Figure 1). The two isotopomers were studied in separate cohorts, in which [benzene ring-U-14C]-pomotrelvir and [lactam carbonyl-14C]-pomotrelvir targeted PBI-0451A (contains benzene but not lactam ring) and S4-Q1 (contains lactam but not benzene ring) and their secondary metabolites, respectively, in addition to providing the assessment of the pharmacokinetics, metabolism and disposition of total radioactivity, parent drug and metabolites generally.

Study Design

This was a Phase 1, open-label, single-center, nonrandomized two-cohort mass balance study conducted in healthy adult males. This study was conducted according to the principles of the International Council for Harmonization (ICH) E6(R2): Good Clinical Practice (GCP). All aspects of the study were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the protocol, and all national, state and local laws or regulations. All participants provided informed consent on an institutional review board–approved protocol.

Eligible participants were confined to the study center from Day -1 to Day 11. On Day 1, the eligible participants were divided equally between the two treatment cohorts (n=8 / cohort) and administered a single oral dose of one of the two isotopomers of [14C]-pomotrelvir as follows, with consumption of a standard low-fat meal.

Lactam cohort (cohort 1): 5 µCi [lactam carbonyl-14C]-pomotrelvir / 700 mg pomotrelvir
Benzene cohort (cohort 2): 5 µCi [benzene ring-U-\(^{14}\)C]-pomotrelvir / 700 mg pomotrelvir

The pharmacokinetics, routes and rates of excretion of pomotrelvir were determined by the assessment of concentrations of total \(^{14}\)C radioactivity in whole blood, plasma, and total recovery in urine and feces. The unlabeled pomotrelvir and two major metabolites PBI-0451A and S4-Q1 in plasma and urine, as well as the percentage of recovery in urine were also evaluated. Blood samples for total \(^{14}\)C determination in whole blood and plasma and unlabeled pomotrelvir determination in plasma were collected at the following time points: pre-dose; and after dosing at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 min, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240 hours, with a return visit collections at 336 h (cohort 1 only). Complete urine collections for unlabeled pomotrelvir and total \(^{14}\)C determination were performed and pooled after dosing at intervals of 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240 hours, and at a return visit of 312-336 hours (cohort 1 only). Additionally, 0.5% of surfactant Triton X-100 was added to the urine samples to prevent pomotrelvir and metabolites from non-specifically binding to the plastic sample containers. Complete feces collections for total \(^{14}\)C determination were performed and pooled after dosing at intervals of 0-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216, 216-240 hours, and at a return visit of 312-336 hours (cohort 1 only).

Safety and Tolerability

Safety and tolerability were assessed throughout the study period by monitoring and recording adverse events, clinical laboratory tests (hematology, serum chemistry, coagulation and urinalysis panels), vital signs (blood pressure, heart rate, respiratory rate and oral temperature), physical examination findings, 12-lead ECG results, and use of concomitant medications and SARS-CoV-2, Hepatitis B virus, Hepatitis C virus, and human immunodeficiency virus-1 (HIV-1) testing was performed at screening.

Determination of Total \(^{14}\)C-Concentrations and Mass Balance

The analysis of radioactivity was performed by Pharmaron Inc. (Germantown, MD). The urine and feces homogenate samples were analyzed by liquid scintillation counting (LSC), where concentrations were above the lower limit of quantitation (LLOQ, as measured by 2x the
measured counts per minute of a scintillant vial with no sample). The whole blood and plasma, urine and feces homogenate samples that had concentrations below the LLOQ of LSC were analyzed for $^{14}$C total radioactivity using a qualified graphitization and AMS assay. Sodium benzoate was employed as a carbon carrier when needed.

Radioactive $^{14}$C recovery in urine and feces was calculated by summation of the amount excreted in each collection interval during the residential period up to 336 hours post dose for the lactam cohort and 240 hours post dose for the benzene cohort.

Analysis of Non-Radiolabeled Pomotrelvir and Metabolites PBI-0451A and S4-Q1
Plasma and urine samples were analyzed for the unlabeled pomotrelvir parent molecule and its hydrolysis metabolites, PBI-0451-A and S4-Q1, using validated (plasma) and qualified (urine) LC-MS/MS methods at QPS, LLC (Newark, DE) or PPD Laboratories (Middleton, WI) after protein precipitation using acetonitrile. Stable isotope labeled internal standards were used for pomotrelvir and PBI-0451-A. S4-Q1 and its analogue internal standard were derivatized with benzoyl chloride prior to analysis. The quantification range for pomotrelvir, PBI-0451A, and S4-Q1 was 5-5000 ng/mL, 5-5000 ng/mL, and 10-10000 ng/mL in plasma, respectively, and 5-5000 ng/mL, 5-5000 ng/mL, and 20-20000 ng/mL in urine.

Pharmacokinetics Analysis
Relevant PK parameters were determined for whole blood and plasma total $^{14}$C radioactivity, unlabeled pomotrelvir parent molecule and its hydrolysis metabolites, PBI-0451A and S4-Q1, in plasma. The analyses were performed using standard noncompartmental methods with Phoenix WinNonlin software (version 8.3; Certara USA Inc., Princeton, NJ). The area under the curve (AUC) parameters were calculated using actual sampling times and the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear-up and log-down method).

Sample Pooling and Preparation for Metabolite Profiling and Characterization
Plasma samples (0-96 h post dose) were pooled across each subject using an AUC approach (Hamilton et al., 1981) that represent ≥ 80% of total AUC according to the measured total
radioactivity, whereas urine and homogenized feces from each subject were pooled based on a ratio of the total weight of the sample excreted at each time point (Penner et al., 2009). After each individual subject pool was prepared, a constant proportion of each subject pool was taken to create one cross-subject pool for plasma, urine and homogenized feces for each cohort (total of 6 pooled samples).

The pooled plasma samples were extracted in three consecutive steps starting with 3 volumes of acetonitrile, followed by 3 volumes of 50% acetonitrile, then 3 volumes of pure water, while the pooled feces homogenates were extracted by two steps of 3 volumes of acetonitrile, followed by 3 volumes of 70% acetonitrile. The supernatants from each extraction steps were combined for plasma or feces samples, which were either directly injected (feces) or further dried and reconstituted in 10% acetonitrile (plasma) for metabolite profiling and structure characterization. The pooled urine samples were centrifuged prior to the metabolite profiling and characterization analysis.

**Metabolite Profiling and Characterization**

For metabolite profiling, [14C] pomotrelvir and metabolites in each of the prepared plasma, feces and urine samples were separated on a Luna Phenyl-Hexyl HPLC column (150 x 4.6 mm, 3 µm; Phenomenex, Torrance, CA) over a 100 min LC elution gradient with the combination of mobile phase A (10mM ammonium acetate) and mobile phase B (100mM ammonium acetate:methanol:acetonitrile (1:8:1 v/v/v)) (gradient: 0 min, 0% B; 3 min, 0% B; 25 min, 10% B; 83 min, 78% B; 93 min, 100% B; 95 min, 100% B; 96 min, 0% B and 100 min, 0% B) at a flow rate of 1.0 mL/min, followed by fraction collection, graphitization, and AMS process (LC-AMS assay).

Following the same LC separation method described above, the metabolite chemical structure characterization analysis was performed using a Q-Exactive mass spectrometer (ThermoFisher Scientific, Bremen, Germany), which was operated in both positive-ion and negative-ion electrospray ionization (ESI) modes, acquiring FTMS (full-scan) data along with tandem mass spectrometric (MS/MS) data using a targeted acquisition list. The identified metabolite structures were confirmed by comparing them to the authentic standards when available.
Results

Clinical Study Subject Disposition
A total of 17 participants were enrolled in the study and 16 participants had qualified data included in the mass balance evaluation, with 1 participant in cohort 2 excluded from analyses due to vomiting within 7 minutes of dosing. All participants were male, with a mean age of 39.8 years (range: 23-54 years). Most participants were Black (70.6%) and not Hispanic or Latino (88.2%), with a mean BMI of 27.14 kg/m² (range: 22.1-31.4 kg/m²). Overall, the type and incidence of AEs was similar across the 2 treatment cohorts. The most frequently reported AEs were somnolence (5 subjects), headache (3 participants), diarrhea (2 participants) and dyspepsia (2 participants) with all other AEs reported for ≤ 1 participant across treatment cohorts. All AEs were mild in severity, except for 2 moderate headaches in 1 participant in the lactam cohort and there were no SAEs or AEs leading to study discontinuation or death reported.

Pharmacokinetics of Total Radioactivity in Plasma and Whole Blood
Figure 2A and 2B present semi-log plots of total radioactivity (converted to equivalent pomotrelvir concentration) in plasma and whole blood up to 336 and 240 hours after a single oral dose of [14C]-pomotrelvir in the lactam and benzene cohorts, respectively. In each treatment cohort, concentration time curves of total radioactivity in plasma and whole blood were similar in shape, with concentrations of total radioactivity consistently higher in plasma compared with whole blood. The blood to plasma ratio did not appear to be time- or concentration-dependent.

For the lactam cohort, the mean maximum [14C] concentration (Cmax) was 5330 ngEq/mL in plasma and 4530 ngEq/mL in whole blood, and the area under the concentration curve from time 0 to the last sampling point (AUClast) was 258 h*µgEq/mL and 247 h*µgEq/mL in plasma and whole blood, respectively. The median time to peak [14C] concentration (Tmax) in plasma and whole blood was 3.5 hours and 2.5 hours, respectively, with a terminal half-life (t1/2) of 88 hours (n=1) in plasma (Table 1). No reliable t1/2 in blood could be estimated. While for the benzene cohort, the mean maximum [14C] Cmax was 11,600 ngEq/mL in plasma and 7680 ngEq/mL in whole blood, and AUClast was 399 h*µgEq/mL and 284 h*µgEq/mL in plasma and whole blood, respectively. The median Tmax in plasma and whole blood was 5.0 hours and 3.5 hours, respectively, with a median terminal half-life (t1/2) of 120 hours in plasma (Table 1). No reliable
t1/2 in blood could be estimated. The high plasma protein binding of pomotrelvir (95.6% plasma protein bound) and some of the metabolites (e.g. PBI-0451A 99.8% plasma protein bound) may contribute to the sustained presence and long T1/2 of the total radioactivity observed in plasma and blood PK profiles.

**Plasma Pharmacokinetics of Pomotrelvir and Its Hydrolysis Metabolites**

The non-radiolabeled pomotrelvir and its two hydrolysis metabolites, PBI-0451A and S4-Q1, were assayed in plasma samples collected from both cohorts using a LC-MS/MS method. As anticipated, the plasma exposure and overall pharmacokinetic profiles of pomotrelvir and its metabolites were similar between the lactam and benzene cohorts (Figure 3A and 3B). As shown in Table 2, the mean pomotrelvir Cmax measured was 2050 ng/mL and 1960 ng/mL, in each cohort, respectively, and the corresponding mean AUCinf (AUC from 0 to extrapolated to infinity) values were 10.8 µg*h/mL and 11.8 µg*h/mL. Median pomotrelvir Tmax in the lactam and benzene cohorts was 0.5 and 0.83 hours, respectively, with median t1/2 14.05 hours in the lactam cohort and 15.51 hours in the benzene cohort.

The pharmacokinetics of the two major hydrolysis metabolites were also evaluated as shown in Table 2. The plasma exposure to PBI-0451A was higher than parent drug with the metabolite to parent (M/P) AUCinf ratios of 3.67 and 4.55 folds in the lactam and benzene cohorts, respectively. On the other hand, the relative AUC0-inf of S4-Q1 was similar to pomotrelvir, with M/P AUCinf ratios of 1.19 in the lactam cohort and 0.98 in the benzene cohort.

**Excretion and Mass Balance**

Following single oral doses of 5 µCi / 700 mg [14C]-pomotrelvir, 93.8% and 94.2% of the radioactivity was recovered in excreta (urine and feces) in lactam and benzene cohorts, respectively (Table 3). The primary route of [14C] excretion was through urine (Figure 4A and 4B). Across the treatment cohorts, 58.2% and 74.9% of the radioactive dose was recovered in urine, and 35.6% and 19.3% of the radioactive dose was recovered in feces, following the lactam-14C- and benzene-14C- labeled pomotrelvir treatments, respectively. In both cohorts, ≥ 80% of total [14C] was recovered within 96 hours after oral dosing.
The radioactivity recovery in urine and feces varied between the two cohorts, which could be explained by the difference in excretion pathways between the two groups of metabolites that were differentially traced by the two radiolabeled isotopomers. As shown in Table 5, for example, M371 and M252, which were traced only in benzene cohort, were mainly eliminated through urine and accounted for near 40% of the total drug dose; while in the lactam cohort, the early elution polar metabolites (LC retention time (RT) at 2.5 min) were excreted in both urine and feces accounting for 24.7% and 11.4% of the total drug dose, respectively.

Renal Excretion of Pomotrelvir and Its Hydrolysis Metabolites

Table 4 summarizes renal PK parameters for pomotrelvir and its metabolites. For both cohorts, the amount of pomotrelvir and its previously known metabolites, PBI-0451A and S4-Q1, excreted through urine accounted for a low fraction, approximately 2%, 3% and 6% of the total dose of 700 mg of pomotrelvir at mean renal clearance (CLr) values of 21, 7.6, 63 mL/min, respectively.

Metabolite Profiling and Characterization

The application of two isotopomers of [14C]-pomotrelvir afforded the opportunity to observe two distinct patterns of LC-radioactivity (AMS) metabolite profiles (Figure 5 and 6) that arose due to the cleavage of the peptide bond in the core structure of pomotrelvir. As such, this approach elucidated numerous metabolites that would have been missed if single labeled position of parent drug was studied. Overall, 11 metabolites were detected that represented > 5% of the total radioactivity in each of the sample analyzed, with 3 metabolites peaks (M167 (S4-Q1), M647, M487) observed only in the samples from the lactam cohort, 6 metabolites peaks (M211, M195, M252, M371, M306 (PBI-0451A) and M482) observed only in the samples from benzene cohort, and 2 metabolites peaks (M471 and M469) observed in both cohorts. All metabolites were identified with chemical structures proposed (Figure 7) based on the fragments in MS/MS spectra (Supplement 1); the structures of M167 (S4-Q1), M195, M306 (PBI-0451A) and M469 were also confirmed with authentic standards by comparing LC retention time and MS/MS
spectra. Table 5 shows the list of metabolites identified in plasma, urine, and feces with the corresponding LC retention times and their relative abundances in each cohort.

In addition, there were drug related components co-eluted at about 2.5 min retention time that could not be quantified due to overlapping elution peaks in the LC-AMS chromatograms in the plasma, urine and feces samples from the lactam cohort (Figure 5) using the reverse-phase LC method. These highly polar metabolites, probably including M181 and M183, were likely formed due to the secondary hydrolysis of the oxidative metabolites of pomotrelvir (e.g., hydrolysis of the oxidative metabolites M469 and M471 to form M181 and M183, respectively).

In plasma over the AUC 0-96 h pool, pomotrelvir represented approximately 7-12% exposure relative to the total radioactivity in the samples. Major metabolites that accounted for > 10% of the total drug-related plasma exposure were the hydrolysis metabolites, including PBI-0451A (34.6%), S4-Q1 (12.7%) and M195 (29.8%), formed by cleavage of either of the two peptide bonds of pomotrelvir. Plasma exposure ratios for PBI-0451A and S4-Q1 to pomotrelvir (M/P; 4.9 and 1.0, respectively) were in line with the M/P results obtained from the bioanalysis of the non-labeled compounds. Drug-related components in excreta that represented > 10% of total drug administered were M371 (18.6%) and M252 (17.7%), which were formed by glucuronidation and glycine conjugation of M195, respectively. Approximately 2% of the pomotrelvir dose was excreted in its intact form in urine, which is consistent with the result from the bioanalysis of the unlabeled pomotrelvir in urine samples. Overall, 3.6% of intact pomotrelvir was recovered in feces.
Discussion

Single oral doses of either of the two radiolabeled isotopomers [lactam carbonyl-$^{14}$C] and [benzene ring-U-$^{14}$C] 5 μCi / 700 mg pomotrelvir solution administered to healthy male adults in this study were safe and well tolerated. Whole blood and plasma PK for total $^{14}$C were similar between the two cohorts. As expected, the PKs of the unlabeled pomotrelvir and its two major metabolites, PBI-0451A and S4-Q1, were similar between the two cohorts and consistent with that observed previously in Phase I clinical studies. The total radioactive dose recovered in urine and feces collected in both cohorts was at about 94%, supporting the quality and validating of this mass balance study in determining the disposition pathways of pomotrelvir. Using this dual-label approach, 11 major metabolites of pomotrelvir were identified and quantified using LC-AMS with chemical structure characterized by LC/MS/MS and confirmed with authentic reference standards for key metabolites of interest.

The absorption of pomotrelvir was rapid with peak concentrations ($C_{\text{max}}$) reached within an hour post dose ($T_{\text{max}}$). Metabolite profiling results of a 0-96 h plasma pool using LC-AMS indicated that the parent drug plasma exposure was approximately 10% (12 and 7% in lactam and benzene cohorts, respectively) of drug-related exposure, while the hydrolysis metabolites M167, M195 and M306 accounted for 13%, 30% and 35% of the total drug related exposure and thus were similar or higher than parent exposure, representing exposure ratios of 1, 4 and 5 versus parent, respectively. Metabolite to parent ratios for previously established hydrolysis metabolites S4-Q1 and PBI-0451A were similar when derived by both the LC-AMS and traditional LC-MS/MS assays, demonstrating the consistency between the two bioanalytical techniques in supporting pomotrelvir analysis.

Whole blood-to-plasma (B/P) total radioactivity AUC$_{\text{last}}$ ratios were <1.0 (0.96 and 0.72 in the lactam and benzene cohorts, respectively) and consistent over the concentration vs time profile, indicating non-preferential distribution of pomotrelvir and its metabolites to blood cells, thus supporting the use of plasma as the appropriate matrix for the assessment of pomotrelvir PK.

With respect to the route of excretion of pomotrelvir, while the majority of radioactivity was recovered in urine for both cohorts, only 2% was excreted as intact drug. The renal clearance of
Pomotrelvir (~20 mL/min) was much lower than glomerular filtration rate (~120 mL/min), which may be attributed by the low free fraction in plasma (4.4%). The overall observations support that pomotrelvir is not actively secreted via renal transporters.

Pomotrelvir was extensively metabolized in humans, leaving only 5-7% pomotrelvir excreted in its intact form after a 700-mg oral dose. Moreover, metabolite profiling showed that the hydrolysis metabolites formed by breaking the peptide bonds of pomotrelvir, PBI-0451A (M306) and M195, that had high relative plasma exposure, accounted only for 8% and 2% of the dose recovered in excreta, respectively, indicating the role of secondary metabolism. The most abundant metabolites in excreta were from glucuronidation (M371) and the glycine conjugation (M252) of the hydrolysis metabolite M195, accounting for 19 and 18% of dose recovered in urine, respectively, whereas the glucuronidation of M306 accounted for 5% of the dose. Oxidative metabolites (M471 and M469) were also observed but with a low abundance of 1-2% found in urine; however, another group of highly polar metabolites (including M181 and M183) showed high abundance in excreta accounting for 25 and 11% of the dose in urine and feces, respectively. These polar metabolites have similar chemical structure to S4-Q1 (M167) but with oxidation on the lactam ring. These metabolites are unlikely due to the oxidation of S4-Q1 itself that showed no metabolism in \textit{in vitro} incubations in human liver microsomes and hepatocytes. Instead, the proposed pathway for these metabolites is primary oxidation on the lactam ring with subsequent peptide bond hydrolysis (e.g. the hydrolysis of the oxidative metabolites M469 and M471 generated the polar metabolites M181 and M183, respectively). These overall results suggest that the predominant biotransformation pathway of pomotrelvir is through hydrolysis of the peptide bonds, that can either be followed by phase II conjugations or occur after phase I oxidation of pomotrelvir.

It is worth noting that, prior to this study, we only knew two major hydrolysis metabolites (PBI-0451A and S4-Q1) based on preliminary metabolite profiling studies, which were conducted using LC-MS/MS method alone, in animals and humans dosed with non-labeled pomotrelvir. Many other significant metabolites, such as the early eluted polar metabolites, M195 and its secondary metabolites were not picked up before. That was due to the challenges in the applied...
LC-MS/MS technique, such as high noise to the early eluting components (polar components), poor ionization efficiency in mass spectrometer (M195) and limited information of metabolism to support target chemical structure search. These metabolites were only revealed in this study with the use of the two radiolabeled isotopomers that allowed the spotting of the metabolites in the metabolite profiling results and provided clues for the elucidation of chemical structures based on which labeled cohort the metabolites were observed. Meanwhile, all these human metabolites were then searched and found in mice, one of the animal species used in preclinical safety and toxicity evaluation for pomotrelvir.

Overall, in this mass balance study, by applying two isotopomers of radiolabeled pomotrelvir with $^{14}$C labeling on separate positions, 11 major metabolites were identified. Among these metabolites, 3 were uniquely observed in the radiolabeled metabolite profiling data for lactam-labeled cohort, and 6 were found only in the benzene-labeled cohort data. These results supported the utility of two isotopomers in providing a comprehensive metabolite profiling and subsequent evaluation of the disposition pathways for pomotrelvir, a compound with complex metabolism, that could be less well characterized using traditional single position radio-labeled material.
Acknowledgments

The authors thank the participants in this study, and the personnel at Pharmaron, QPS, LLC, and PPD Laboratories who were involved in the conduct of this study, and Pardes colleagues for editorial support.

Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.

Authorship Contributions

Participated in research design: Yang, Wilfret, Turnquist, Plummer, van Ingen, Kearney
Conducted experiments: Yang, Cha, Plummer
Performed data analysis: Wong, Yang, Wilfret, van Ingen
Wrote or contributed to the writing of the manuscript: Wong, Yang, Wilfret, Turnquist, Kearney
References


Figure Legends.

Figure 1. Chemical structures of the two radiolabeled pomotrelvir isotopomers. *Labeling position. A. [lactam carbonyl-$^{14}$C]PBI-0451; B. [benzene ring-$^{14}$C]PBI-0451

Figure 2. Mean (±SD) total [$^{14}$C] (converted to equivalent pomotrelvir concentration) in plasma and whole blood following a single oral dose of 5 µCi/700 mg [$^{14}$C]-pomotrelvir. A. Lactam cohort; B. Benzene cohort.

Figure 3. Mean (± SD) concentration-time plots of plasma pomotrelvir and metabolites PBI-0451-A and S4-Q1 (Semilogarithmic Scales). A Lactam cohort, B Benzene cohort.

Figure 4. Mean (±SD) cumulative percent recovery of total radioactivity in urine and feces following a single oral dose of 5 µCi/700 mg [$^{14}$C]-Pomotrelvir. A. Lactam cohort; B. Benzene cohort.

Figure 5. LC-radioactivity (LC-AMS) metabolite profiles in plasma (0–96-h pool), urine (0–336-h pool), and feces (0–336-h pool) following a single oral dose of 5 µCi/700 mg [lactam carbonyl-$^{14}$C]-pomotrelvir in healthy male adults

Figure 6. LC-radioactivity (LC-AMS) metabolite profiles in plasma (0–96-h pool), urine (0–336-h pool), and feces (0–336-h pool) following a single oral dose of 5 µCi/700 m [benzene ring-$^{14}$C]-pomotrelvir in healthy male adults

Figure 7. Proposed metabolite scheme for pomotrelvir following administration of a single oral dose of 700 mg of $^{14}$C-pomotrelvir solution in healthy male volunteers.
Table 1. Mean (%CV) Total Radioactivity Pharmacokinetic Parameters in Plasma and Whole Blood

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Total Radioactivity</th>
<th>Lactam Cohort (n = 8)</th>
<th>Benzene Cohort (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma</td>
<td>Whole Blood</td>
<td>Whole Blood/Plasma</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ngEq/mL)</td>
<td>5330 (15.6) n = 8</td>
<td>4530 (23.5) n = 8</td>
<td>0.846 (13.8) n = 8</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\text{last}}$ (h*µgEq/mL)</td>
<td>258 (23.8) n = 8</td>
<td>247 (23.2) n = 8</td>
<td>0.960 (5.9) n = 8</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\text{inf}}$ (h*µgEq/mL)</td>
<td>240 (NC) n = 1</td>
<td>NC</td>
<td>NA</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)$^a$</td>
<td>3.50 (1.50, 12.00) n = 8</td>
<td>2.50 (1.00, 8.00) n = 8</td>
<td>NA</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)$^a$</td>
<td>88.1 (88.1, 88.1) n = 1</td>
<td>NC</td>
<td>NA</td>
</tr>
</tbody>
</table>

CV = coefficient of variation; PK = pharmacokinetic(s); NC = not calculated; NA = not applicable.

$^a$ Values reported are median (min, max).
Table 2: Mean (%CV) Plasma Pharmacokinetic Parameters for Pomotrelvir and Metabolites PBI-0451-A and S4_Q1

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Lactam Cohort (n = 8)</th>
<th></th>
<th>Benzene Cohort (n = 8)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pomotrelvir</td>
<td>PBI-0451-A</td>
<td>S4_Q1</td>
<td>Pomotrelvir</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>2050 (35.8)</td>
<td>1690 (18.8)</td>
<td>226 (13.7)</td>
<td>1960 (16.9)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; (µg*h/mL)</td>
<td>10.70 (24.20)</td>
<td>26.10 (34.3)</td>
<td>4.16 (18.9)</td>
<td>11.60 (29.1)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-inf&lt;/sub&gt; (µg*h/mL)</td>
<td>10.80 (23.8)</td>
<td>26.30 (34.1)</td>
<td>4.40 (17.0)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.80 (28.5)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50 (0.50, 1.00)</td>
<td>5.00 (2.00, 6.00)</td>
<td>6.00 (4.00, 8.00)</td>
<td>0.83 (0.52, 3.00)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.05 (12.18, 17.06)</td>
<td>14.79 (12.70, 20.52)</td>
<td>12.98 (9.36, 22.34)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.51 (13.65, 17.13)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; M/P ratio</td>
<td>—</td>
<td>1.34 (33.0)</td>
<td>0.330 (34.3)</td>
<td>—</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; M/P ratio</td>
<td>—</td>
<td>3.67 (27.1)</td>
<td>1.10 (20.5)</td>
<td>—</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-inf&lt;/sub&gt; M/P ratio</td>
<td>—</td>
<td>3.64 (27.2)</td>
<td>1.19 (27.5)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
</tr>
</tbody>
</table>

CV = coefficient of variation; M/P = metabolite/parent corrected for molecular weight; PK = pharmacokinetic(s)

<sup>a</sup> Data for n = 7 subjects.
<sup>b</sup> Values reported are median (min, max).
Table 3. Total Recovery of the Radioactivity in Urine and Feces Following a Single Oral Dose of 5 µCi/700 mg [¹⁴C]-Pomotrelvir

<table>
<thead>
<tr>
<th>Recovery%</th>
<th>Lactam Cohort (n=8)</th>
<th>Benzene Cohort (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
</tr>
<tr>
<td>Mean (CV%)</td>
<td>58.2 (11.7)</td>
<td>35.6 (22.0)</td>
</tr>
</tbody>
</table>
Table 4. Mean (%CV) Renal Excretion Parameters for Pomotrelvir and Metabolites PBI-0451A and S4-Q1

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Lactam Cohort (n = 8)</th>
<th>Benzene Cohort (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pomotrelvir</td>
<td>PBI-0451A</td>
</tr>
<tr>
<td>%(\text{f}_{\text{ex}}) (CV%)</td>
<td>1.78 (24.1)</td>
<td>2.20 (16.0)</td>
</tr>
<tr>
<td>CL(_r) (mL/min) (CV%)</td>
<td>18.8 (28.6)</td>
<td>7.2 (36.0)</td>
</tr>
</tbody>
</table>

CL\(_r\) = renal clearance; CV = coefficient of variation; PK = pharmacokinetic(s)
Mean (%CV) was calculated from cumulative values through 336 hours for the lactam cohort and 240 hours for the benzene cohort.
Table 5. Pomotrelvir and Its Metabolites Identified in Plasma, Urine and Feces Following a Single Oral Dose of 700 mg of $^{14}$C-Pomotrelvir (5 µCi) Solution in Healthy Male Adults

<table>
<thead>
<tr>
<th>Componentsa</th>
<th>LC RT (min)</th>
<th>Plasma% of total drug-related exposure</th>
<th>Urine% of dose</th>
<th>Feces% of dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactam Cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixture (proposed mainly M181, M183), hydrolysis</td>
<td>2.5</td>
<td>20.8</td>
<td>24.7</td>
<td>11.4</td>
</tr>
<tr>
<td>M167: S4-Q1, hydrolysis</td>
<td>15.2</td>
<td>12.7</td>
<td>6.36</td>
<td>0.73</td>
</tr>
<tr>
<td>M647: Glucuronidation of monohydroxy of parent</td>
<td>63.1</td>
<td>5.11</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M487: oxidation (+ 2O)</td>
<td>65.2</td>
<td>3.34</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M471: monohydroxy</td>
<td>73.6</td>
<td>7.79</td>
<td>1.52</td>
<td>1.31</td>
</tr>
<tr>
<td>M469: monohydroxy-2H</td>
<td>75.2</td>
<td>4.76</td>
<td>1.93</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M455: parent</td>
<td>77.2</td>
<td>12.4</td>
<td>2.25</td>
<td>5.07</td>
</tr>
<tr>
<td><strong>Benzene Cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M211: oxidation of M195</td>
<td>12.3</td>
<td>2.15</td>
<td>3.53</td>
<td>3.95</td>
</tr>
<tr>
<td>M195: hydrolysis</td>
<td>41.0</td>
<td>29.8</td>
<td>&lt;1</td>
<td>1.79</td>
</tr>
<tr>
<td>M371: Glucuronidation of M195</td>
<td>42.6</td>
<td>&lt;1</td>
<td>18.6</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M252: Glycine conjugation of M195</td>
<td>45.3</td>
<td>3.42</td>
<td>17.7</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M306: PBI-0451A, hydrolysis</td>
<td>61.8</td>
<td>34.6</td>
<td>7.66</td>
<td>0.70</td>
</tr>
<tr>
<td>M482: Glucuronidation of M306</td>
<td>63.1</td>
<td>1.12</td>
<td>5.11</td>
<td>&lt;1</td>
</tr>
<tr>
<td>M471: monohydroxy</td>
<td>73.6</td>
<td>2.04</td>
<td>1.61</td>
<td>1.08</td>
</tr>
<tr>
<td>M469: monohydroxy-2H</td>
<td>75.2</td>
<td>3.95</td>
<td>1.93</td>
<td>0.46</td>
</tr>
<tr>
<td>M455: parent</td>
<td>77.2</td>
<td>6.99</td>
<td>2.59</td>
<td>2.28</td>
</tr>
</tbody>
</table>

*a: Components > 5% of the total radioactivity in each sample, but not limited to, are listed.
b: Plasma 0-96 h AUC pool for both lactam and benzene cohorts
c: Urine 0-336 h pool for lactam cohort and 0-240 h for benzene cohort
d: Feces 0-336 h pool for lactam cohort and 0-240 h for benzene cohort
LC RT: Liquid chromatography retention time

The metabolites and parent were named according to their molecular weight, e.g. M167 metabolite has molecular weight of 167.
**Figure 1**

A

![Chemical structure A]

B

![Chemical structure B]
Figure 2

A

B
Figure 3

A

B
Figure 4

A

![Graph A]

B

![Graph B]
Figure 5

Plasma, 0-96 h
Polar metabolites

Urine, 0-336 h
Polar metabolites

Feces, 0-336 h
Polar metabolites
Figure 6

- **Plasma, 0-96 h**
  - Activity, DPM
  - M211
  - M195
  - M252
  - M471
  - Pomotrelvir

- **Urine, 0-240 h**
  - Activity, DPM
  - M211
  - M371
  - M252
  - M482
  - M471
  - Pomotrelvir

- **Feces, 0-336 h**
  - Activity, DPM
  - M211
  - M195
  - M469
  - M471
  - Pomotrelvir
Characterization of Pharmacokinetics, Biotransformation and Elimination of Pomotrelvir Orally Administered in Healthy Male Adults Using Two [14C]-labeled Microtracers with Separate Labeling Positions

Authors: Ziping Yang, Shekman L Wong, David Cha, David Wilfret, David Turnquist, Andrew Plummer, Eric van Ingen, Brian P. Kearney

Journal: Drug Metabolism and Disposition
Manuscript #: DMD-AR-2023-001439

Supplement 1

Metabolite Structure Characterization from MS/MS Spectra

Note: The following depicted MS/MS data represent product ion spectra obtained by collision-induced dissociation of the respective pomotrelvir or its metabolite parent ions – MH+ (positive ionization mode) or M-H (negative ionization mode).
Parent compound: pomotrelvir (MW: 455)

\[ \text{H}^+ \]
Calculated m/z 456.17969

Calculated m/z 289.07383
-NH3 Calculated m/z 151.08659

Calculated m/z 261.07892

Calculated m/z 168.11314

Calculated m/z 141.10224

Calculated m/z 84.08078

Calculated m/z 196.10805

Calculated m/z 262.15424

Calculated m/z 439.14780

Calculated m/z 456.17758
M167: S4-Q1, hydrolysis

\[ \text{H}^+ \] Calculated m/z 168.11314
-NH3 Calculated m/z 151.08659

Calculated m/z 141.10196
-NH3 Calculated m/z 124.07553

Calculated m/z 141.10224
-NH3 Calculated m/z 124.07569

Calculated m/z 168.11284
M647: Glucuronidation of mono-hydroxy of parent

$^+H$  Calculated m/z 648.20670
M482: Glucuronidation of M306

- $\text{H}^+$ Calculated $m/z$ 483.11648
- $\text{-H}_2\text{O}$ Calculated $m/z$ 465.10592
- $\text{-2H}_2\text{O}$ Calculated $m/z$ 447.09536
- $\text{GluA}$ Calculated $m/z$ 307.08440
- $\text{GluA, -H}_2\text{O}$ Calculated $m/z$ 289.07383

Calculated $m/z$ 261.07892
Calculated $m/z$ 178.00481
Calculated $m/z$ 84.08118

$\text{GluA}$
M487: oxidation (+ 2O)

Calculated m/z 488.16952

Calculated m/z 289.07383
Calculated m/z 261.07892
Calculated m/z 200.10297
Calculated m/z 228.09788
Calculated m/z 294.14483
Calculated m/z 178.00542
M471: monohydroxy

- H^+ Calculated m/z 472.17461
- H2O Calculated m/z 454.16404

Calculated m/z 289.07383
Calculated m/z 261.07892
Calculated m/z 178.00542

- H2O Calculated m/z 166.09749
- H2O Calculated m/z 139.08659
- H2O Calculated m/z 194.09240
- H2O Calculated m/z 260.13935

Calculated m/z 84.08078

Relative Abundance

Calculated m/z 472.17461
Calculated m/z 454.16404
Calculated m/z 289.07383
Calculated m/z 261.07892
Calculated m/z 178.00542

- H2O Calculated m/z 166.09749
- H2O Calculated m/z 139.08659
- H2O Calculated m/z 194.09240
- H2O Calculated m/z 260.13935

Calculated m/z 84.08078

Relative Abundance
M469: monohydroxy-2H

Calculated m/z 470.15896

Calculated m/z 289.07383
Calculated m/z 210.08732
Calculated m/z 182.09240
Calculated m/z 261.07892
Calculated m/z 178.00542
Calculated m/z 84.08078

Relative Abundance

H+
M306: PBI-0451A, hydrolysis

$\text{H}^+$ Calculated $m/z$ 307.08440

Calculated $m/z$ 261.07892
Calculated $m/z$ 178.00542
Calculated $m/z$ 84.08078

Relative Abundance

84.08112
67.05463
106.64685
152.02554
195.03168
218.07317
261.07816
307.08356
M183: oxidation, hydrolysis

-2NH, -H2O Calculated m/z 136.07569

-H⁺ Calculated m/z 184.10805

-N Calculated m/z 114.06753
M195: hydrolysis

Calculated $m/z$ 194.00143

-Calculated $m/z$ 150.01160
- $-\text{HCl}$ Calculated $m/z$ 114.03492

$\text{H}^+$
M252: Glycine conjugation of M195

$\text{H}^+$ Calculated $m/z$ 251.02289

Calculated $m/z$ 150.01160
-HCl Calculated $m/z$ 114.03492

Calculated $m/z$ 207.03306
M371: Glucuronidation of M195

- Calculated m/z 370.03352
- GluA Calculated m/z 194.00143
Typical product ion fragments for pomotrelvir and its associated metabolites, and their chemical formulars and theoretical accurate mass values

![Chemical structures](image-url)