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## **Title: Metabolism and Disposition of Hepatitis C Polymerase Inhibitor**

### **Dasabuvir in Humans**

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**Running Title**

**a) Metabolism and disposition of [<sup>14</sup>C]dasabuvir in humans.**

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**d) ABBREVIATIONS:**

HCV, hepatitis C virus; DAAs, direct-acting antiviral agents; AUC, area under the curve; CID, collisional induced dissociation; LSC, liquid scintillation counting; HPLC, high-performance liquid chromatography; SPE, solid phase extraction; M1, N-(6-(5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(1-hydroxy-2-methylpropan-2-yl)-2-methoxyphenyl)naphthalen-2-yl)methanesulfonamide; M5, 2-(5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-methoxy-3-(6-(methylsulfonamido)naphthalen-2-yl)phenyl)-2-methylpropanoic acid; BID, twice a day; CYP, cytochrome P450; FMO, flavin-containing monooxygenase.

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### Abstract

Dasabuvir (also known as ABT-333), a potent non-nucleoside NS protein 5B polymerase inhibitor of the hepatitis C virus (HCV), is being developed in combination with paritaprevir / ritonavir and ombitasvir 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 dasabuvir in humans. Following the administration of a single 400-mg oral dose of [<sup>14</sup>C]dasabuvir, without co-administration of paritaprevir / ritonavir and ombitasvir, to four healthy male volunteers the mean total percentage of the administered radioactive dose recovered was 96.6%. The recovery from the individual subjects ranged from 90.8 to 103%. Dasabuvir and corresponding metabolites were predominantly eliminated in feces (94.4% of dose), and minimally through renal excretion (2.2% of dose). The biotransformation of dasabuvir primarily involves hydroxylation of the *tert*-butyl group to form active metabolite M1, followed by glucuronidation and sulfation of M1 and subsequent secondary oxidation. Dasabuvir was the major circulating component (58% of total radioactivity) in plasma, followed by metabolite M1 (21%). Other minor metabolites represented less than 10% each of total circulating radioactivity. Dasabuvir was cleared mainly through CYP-mediated oxidation metabolism to M1. M1 and its glucuronide and sulfate conjugates were primarily eliminated in feces. Subsequent oxidation of M1 to the *tert*-butyl acid followed by formation of the corresponding glucuronide conjugate plays a secondary role in elimination. Cytochrome P450 profiling indicated that dasabuvir was mainly metabolized by CYP2C8 followed by CYP3A4. In summary, the biotransformation pathway and clearance routes of dasabuvir were characterized, and the structures of metabolites in circulation and excreta were elucidated.

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### Introduction

HCV is a virus that affects approximately 150 million people worldwide (WHO, 2015). The virus, which replicates predominantly in the cytoplasm of hepatocytes, can lead to acute or chronic liver infections that introduce the likelihood of developing liver cirrhosis or cancer.

Dasabuvir, also known as ABT-333, is a nonstructural protein 5B (NS5B) inhibitor that has been developed for the HCV genotype-1 infection in combination with an NS3 protease inhibitor paritaprevir with ritonavir (r) and/or an NS5A non-nucleoside polymerase inhibitor (ombitasvir) with or without ribavirin (RBV) (Maring et al., 2009, Feld et al., 2014; Ferenci et al., 2014; Kowdley et al., 2014; Zeuzem et al., 2014). RNA polymerase (NS5B) is a key element in the replication of HCV utilizing an atypical ability of initiating RNA synthesis without using an RNA primer (Moradpour et al., 2007; Rigat et al., 2010, Beaulieu, 2009). Non-nucleoside inhibitors commonly interrupt the start of the RNA synthesis phase (Legrand-Abravanel et al., 2010). Dasabuvir exhibits potent inhibition against genotype 1a and 1b HCV polymerases ( $IC_{50}$  2.2 – 10.7 nM in a biochemical enzymatic assay) and against genotype 1a ( $EC_{50}$  7.7 nM) and 1b ( $EC_{50}$  1.8 nM) HCV replicons (Maring et al., 2009).

Clinically, dasabuvir has favorable safety, tolerability and pharmacokinetic profiles as a monotherapy or combination therapy at doses administered to date. Minimal to no accumulation was observed from dasabuvir at doses ranging from 200 mg BID to 600 mg BID. A modest accumulation of 65% was observed following a 1000 mg BID dose regimen of dasabuvir and approximately 2-fold accumulation was observed when dosing above 1000 mg BID. The absolute bioavailability of the dasabuvir 400 mg tablet was 46% compared to an intravenous microdose of approximately 85  $\mu$ g of [ $^{14}$ C]dasabuvir administered at the same time as the oral

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dose. According to dasabuvir Phase 1 studies, when the non-radiolabeled dasabuvir was administered as a single agent, the dasabuvir M1 metabolite-to-parent ratio was around 0.35. This report describes the absorption, metabolism, and excretion (AME) of a single 400-mg oral dose of [<sup>14</sup>C]dasabuvir in four healthy human subjects. The purposes of the study are to assess the mass balance, elucidate the routes and rates of excretion, identify and quantify the exposure of circulating metabolites in human plasma, elucidate the metabolite structures, determine the metabolite profiles in excreta and understand the metabolic pathway of dasabuvir in humans. In addition, *in vitro* characterization of the major drug metabolism enzymes which are responsible for the metabolism of dasabuvir is also described.

## Materials and Methods

### Drugs and Reagents

Dasabuvir (N-(6-(3-(*tert*-butyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-methoxyphenyl)naphthalen-2-yl)methanesulfonamide), its *tert*-butyl hydroxyl metabolite (M1) and *tert*-butyl carboxylate metabolite (M5), [<sup>14</sup>C]dasabuvir and [<sup>3</sup>H]dasabuvir were supplied by Process Chemistry, AbbVie, Inc (North Chicago, IL). The structures of dasabuvir and its metabolite standards are shown in Fig. 1. The radiochemical synthesis of [<sup>14</sup>C]dasabuvir was conducted in two steps using (2-[<sup>14</sup>C]) uracil as the radiolabeled starting material. Purification of the compound by crystallization provided >99% radiochemical purity by HPLC. [<sup>3</sup>H]Dasabuvir was prepared via reduction of 5-bromouracil dasabuvir with tritium for *in vitro* assays. The radiochemical purity after HPLC purification was >99%. Metabolite standards were as follows: N-(6-(5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(1-hydroxy-2-methylpropan-2-yl)-2-methoxyphenyl)naphthalen-2-yl)methanesulfonamide (M1); 2-(5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2-methoxy-3-(6-(methylsulfonamido)naphthalen-2-yl)phenyl)-2-

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methylpropanoic acid (M5). These reference standards were used as HPLC and mass spectrometric standards.

### 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 [<sup>14</sup>C]dasabuvir under non-fasting conditions. The study drug, [<sup>14</sup>C]dasabuvir (400 mg active, 100 μCi [<sup>14</sup>C]) was administered as a liquid suspension. 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. Subjects were released from the study site at any time after 120 hours post dose if the preset release criteria were met.

Blood samples were collected by venipuncture into vacutainer collection tubes containing potassium EDTA at the following times: 0 hour (predose), 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240 hours after dosing of [<sup>14</sup>C]dasabuvir on day 1 of the study. 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, 192 to 216, 216 to 240 hours after dosing of [<sup>14</sup>C]dasabuvir on Study Day 1. Urine samples were spiked with dodecylbenzenesulfonic acid (DBSA) sodium salt at a concentration of 0.6 mg/mL prior to sample aliquoting. Aliquots of the urine were frozen and maintained at -20°C prior to metabolite profiling.

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Fecal samples were collected pre-dose (upon check-in before dosing) 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, 192 to 216, and 216 to 240 hours. All feces collected during a collection interval were kept frozen at  $-20^{\circ}\text{C}$  prior to metabolite profiling.

### **Total Radioactivity Measurement by Liquid Scintillation Counting**

All sample combustion was performed using a Model 307 Sample Oxidizer (Packard Instrument Company) and the resulting  $^{14}\text{CO}_2$  was trapped in a mixture of Perma Fluor and Carbo Sorb. The efficiency of the oxidizer was evaluated each day 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 and an aliquot was mixed with scintillation cocktail before radioanalysis. All samples were analyzed in duplicate if the sample size allowed unless the entire sample was used for analysis. If results from sample replicates (calculated as  $^{14}\text{C}$  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.

After mixing, duplicate blood samples were weighed (approximately 0.2 g), combusted, and analyzed by LSC. The representative lower limit of quantitation for blood was 195 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

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173 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 160 ng equivalents/g. Fecal samples were combined by subject 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 room temperature and pooled across subjects at selected time points in addition to AUC plasma pooling utilizing the Hamilton method (Hamilton et al., 1981) for each subject. The pooled plasma was processed using the following protein precipitation method. In brief, a six-fold volume of acetonitrile/methanol (3:1, v/v) was added to each sample, followed by vortexing and 5 min sonication. The sample was then centrifuged at 3500 rpm (2465 x g) at 4°C. The supernatant was transferred a glass tube. The protein pellets were further washed with 3-5 mL of acetonitrile-methanol (4:1, v/v), followed by centrifugation (3500 rpm, 2465 x g). After combining the supernatants 50 µL of DMSO was added and the solution was concentrated to ~50-100 µL volume under a stream of nitrogen. The remaining material was diluted with 150 µL of 0.1% formic acid in water. An aliquot of the reconstituted sample was subjected to LSC counting to determine total radioactivity recovery. Another aliquot of the reconstituted sample was transferred to an HPLC autosampler vials and 75 µL of the reconstituted sample was injected for LC-MS and radiochromatographic analysis.



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Urine samples were pooled at selected time points across subjects. Pooled samples were processed using solid phase extraction (Agilent Accu-Bond II Octyl SPE cartridge, 1000 mg/6 mL, PN188-0360). In brief, the SPE cartridges were conditioned with acetonitrile (2x bed volume), methanol (2x) and 0.1% formic acid-water (4x) before use. Aliquots of urine were loaded to pre-conditioned columns, washed with water (4x bed volume), and eluted with 3 x 4 mL acetonitrile/methanol (3:1 v/v). The eluents were combined followed by addition of 100  $\mu$ L DMSO. The combined eluents were then concentrated under a stream of nitrogen to about 100-200  $\mu$ L followed by dilution of the sample with 250  $\mu$ L of 0.1% aqueous formic acid before LC-MS analysis.

Fecal homogenate samples were pooled at each time point across subjects before processing. The fecal samples were processed using multiple solvent extractions with acetonitrile-methanol (3:1 v/v) using 1:3 sample:solvent ratio, followed by centrifugation at 4000 rpm for 20 min at 4°C. The extraction was repeated until either 80% of the radioactivity had been recovered or less than 1% of the radioactivity was extracted. Aliquots of extracted samples were subjected to LSC counting for total radioactivity. A 50  $\mu$ L aliquot of DMSO was added to the combined supernatants before concentrating the sample under a nitrogen flow at room temperature. The final residues were reconstituted with 0.1% formic acid-acetonitrile (1:1) before HPLC-MS-radio analysis.

### **Method for Metabolite Profiles and Identification**

HPLC separation of dasabuvir and the corresponding metabolites was conducted using a Thermo Accela HPLC system (Thermo Fisher, San Jose, CA), which consisted of a Thermo Accela autosampler, 1250 Series binary pump and Accela PDA detector. Separation was achieved on an

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Agilent Eclipse XDB-C18, 3.5  $\mu\text{m}$ , 4.6 x 150 mm column (Agilent Technologies, Santa Clara, CA). The HPLC mobile phase consisted of 0.1% formic acid in water (solvent A), and 100% acetonitrile (solvent B). The HPLC flow rate was 1.0 ml/min. The gradient was as follows: 0-3 min: 20% B; 3-10 min: 20%-40% B; 10-50 min: 40%-63% B; 50-52 min: 63%-95% B; 52-58min: 95% B; 58-59 min: 95% -20% B; 59-65 min: 20% B. The HPLC system was interfaced with a Thermo Fisher Orbitrap Discovery<sup>TM</sup> mass spectrometer (Thermo Fisher Scientific). The mass spectrometric analyses were conducted using electrospray ionization (ESI) operating in negative ionization mode. The MS settings were as follows: ESI voltage: -2.6 kV, capillary temperature 275 °C, capillary voltage 35 V and tube lens 110 V. 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 MS<sup>2</sup> and MS<sup>3</sup>. The mass resolution was set at 30000 for full scan and 7500 for MS<sup>2</sup> and MS<sup>3</sup> scans. Accurate mass measurements were obtained after performing a 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 to collect fractions at intervals of 0.3 min/well. Radioactivity counting was conducted using a Perkin Elmer TopCount NXT system.

### **Quantitation of Dasabuvir and M1 in Plasma**

Plasma concentrations of dasabuvir and metabolite M1 were determined using a validated LC-MS/MS bioanalytical method. A common protein-precipitation procedure followed by on-line

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solid phase extraction (SPE) using a small aliquot of plasma combined with an aliquot of stable-isotope labeled internal standard solution in acetonitrile was utilized in all validated methods. After centrifugation, an aliquot of the supernatant was injected onto the on-line SPE LC-MS/MS system. The chromatographic separation of dasabuvir, M1 and internal standards from the co-extracted matrix components was achieved with a reverse-phase analytical column. Analysis was performed on an AB Sciex triple quadrupole mass spectrometer with a Turbo Ion Spray interface. Detection was performed in the multiple reaction monitoring (MRM) mode at  $m/z$  494.2  $\rightarrow$  359.2 for dasabuvir,  $m/z$  498.2  $\rightarrow$  363.2 for  $^{13}\text{CD}_3$ -dasabuvir (the internal standard of dasabuvir),  $m/z$  510.4  $\rightarrow$  412.4 for M1, and  $m/z$  516.4  $\rightarrow$  418.4 for stable isotope labeled internal standard of M1.

### **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, [ $^{14}\text{C}$ ]dasabuvir, 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, [ $^{14}\text{C}$ ]dasabuvir and the M1 metabolite in plasma were also calculated.

### ***In Vitro* Metabolism and P450 Phenotyping.**

Studies to identify the P450 isoform(s) responsible for formation of M1 were conducted by incubation of the substrate in the presence of recombinant P450 isoforms. Chemical inhibition

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was studied using selected inhibitors in the human liver microsomal preparations. Studies with recombinant human P450 isoforms were conducted using CYP isoforms 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, and FMO isoforms FMO1, FMO3, FMO5, all of which were obtained from BD Bioscience (San Jose, CA). Standard 200  $\mu$ L incubations were performed in duplicate with NADPH (1 mM), 0.25  $\mu$ M of [ $^3$ H]dasabuvir, 50 mM phosphate buffer (pH 7.4), and recombinant human P450 (20 pmol). Reactions were initiated with the addition of an NADPH solution to the incubation mixture and the incubation was conducted at 37 °C. At 0 and 60 min, incubations were quenched with 2x volume of acetonitrile/methanol mixture (1:1, v/v). Quenched samples were centrifuged at 1900g for 5 minutes at 4°C. An aliquot of the supernatant was analyzed using HPLC with radiochemical detection.

Chemical inhibition studies were conducted using human liver microsomes with the following inhibitors of specific P450 isoforms: ketoconazole (CYP3A4/5), quercetin (CYP2C8), quinidine (CYP2D6), and 2-phenyl-2-(1-piperidinyl)propane (CYP2B6). Inhibitor concentrations were selected to bracket the inhibitor  $K_i$ . The inhibitor  $K_i$  used was 0.0037, 0.027, 1.1 and 5.6  $\mu$ M for ketoconazole, quinidine, quercetin, and 2-phenyl-2-(1-piperidinyl)propane, respectively. A standard 0.2 mL incubation contained 0.5 mg/mL human liver microsomes (In Vitro Technologies), 0.25  $\mu$ M [ $^3$ H]dasabuvir and selected concentrations of chemical inhibitors (ketoconazole (0.03 -1  $\mu$ M), quinidine (0.01 -2  $\mu$ M), quercetin (0.5 – 45  $\mu$ M), and 2-phenyl-2-(1-piperidinyl)propane (10-150  $\mu$ M)) in 50 mM phosphate buffer (pH 7.4). After a 5 minute preincubation at 37°C, the reaction was initiated with the addition of NADPH for a final concentration of 1 mM and incubated in a water bath at 37°C. After 0 and 60 min the incubations were quenched with an equal volume of acetonitrile/methanol (1:1, v/v). The

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samples were then centrifuged at 3500 x g for 30 minutes at 4°C. The supernatants were analyzed by HPLC with radiochemical detection.

## Results

### Excretion of Radioactivity

Following a single oral dose of [<sup>14</sup>C]dasabuvir (400 mg, 100 µCi) to four healthy, male volunteers, the excretion of radioactivity in urine and feces from all the subjects was measured over a period of up to 240 hours post dose. Fig. 2 presents the mean cumulative recovery of total radioactivity in excreta expressed as percentage of dose. The overall mean recovery of radioactivity in urine and feces samples was 96.6% (± 5.1%) over the 240 hour collection period, with recovery in individual subjects ranging from 90.8 to 103%. The radioactivity was excreted primarily through fecal elimination (mean, 94.4% of dose). Renal excretion was relatively minor (mean, 2.2% of dose).

### Pharmacokinetic Data Analysis

The pharmacokinetic parameters for dasabuvir, M1, 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 dasabuvir and metabolite M1 were determined using a validated LC-MS/MS bioanalytical method, expressed as ng/mL. The  $T_{max}$  for dasabuvir, M1, and total radioactivity occurred approximately 3-4 hours following single oral dose. Mean peak plasma concentrations ( $C_{max}$ ) for the parent drug, M1, and total radioactivity were 658, 267 ng/mL and 1300 ng-eq/g, respectively. The concentrations of dasabuvir and total radioactivity apparently declined in parallel after reaching the peak concentration (Fig. 3). The  $AUC_{0-last}$  for the parent drug, M1 and total radioactivity were 6260, 2230 ng•h/mL and 8251 ng-eq•h/g,

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respectively. The sum of dasabuvir and M1 exposures measured by the LC-MS bioanalytical method matches reasonably well with the total plasma radioactivity exposure, indicating that dasabuvir and M1 are the primary species in plasma.

### Metabolite Profiles of [<sup>14</sup>C]Dasabuvir in Excreta and Circulation

**Plasma.** A representative HPLC radiochromatogram of [<sup>14</sup>C]dasabuvir and its metabolites in pooled human plasma using Hamilton method (t = 0-12 h postdose) is shown in Fig. 4. The relative amounts of dasabuvir and metabolites in human plasma, expressed as percent of radioactivity in plasma, are summarized in Table 2. [<sup>14</sup>C]Dasabuvir is the predominant component in human plasma, representing 58.1% of total radioactivity in circulation. M1 is the most significant metabolite, accounting for 21.4% of radioactivity, followed by minor metabolites M2, M3, M4, M5, and M6.

**Urine and feces.** The representative HPLC radiochromatogram of pooled human urine is shown in Fig. 5(A). Unchanged parent drug and a total of nine metabolites, including M1-M7, M11 and U1, were detected in human urine. All these metabolites were present at trace or low levels with respect to the administered dose; M1 was the most significant component in urine, accounting for 0.85% of dose.

The representative HPLC radiochromatogram of pooled human feces is shown in Fig. 5(B). M1 was the most abundant radiochemical component in feces, accounting for 31.5% of total dose, followed by unchanged parent drug dasabuvir (26.2%), M2 (15.2%), and M5 (11.1%). Minor metabolites M8, M9 and M10 were also detected in feces, each representing <5% of dose. The mean quantification results for dasabuvir and corresponding metabolites in urine and feces,

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expressed as the percentages of the administered radioactive dose, are tabulated in Table 3. The proposed metabolic scheme for dasabuvir 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 dasabuvir were characterized using a combination of negative ionization high resolution full scan MS and product ion scan (MS/MS) analyses. The structures of the metabolite M1 and M5 were confirmed against the synthesized materials, 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 dasabuvir and metabolites are listed in Table 4. The CID spectrum and detailed assignment of the fragments of dasabuvir and metabolites are provided in the Supplemental Data.

Dasabuvir yielded a deprotonated molecular ion at  $m/z$  492.1601 (calculated mass  $m/z$  492.1599) ( $[M-H]^-$ ) in negative ion mode. Collisional induced dissociation (CID) of the  $m/z$  492 ion gave the characteristic MS/MS fragment ions at  $m/z$  477.1372 (base peak), 462.1137, 449.1543, 435.0897, 414.1825 and 399.1590 (Table 4). The fragment ions at  $m/z$  477.1372 and 462.1137 were the results of loss of one and two methyl groups, respectively. The fragment ion at  $m/z$  435.0897 was derived from a loss of *tert*-butyl group. Minor fragment ions were also observed at  $m/z$  414.1825 (loss of  $CH_2SO_2$ ) and  $m/z$  399.1590 (loss of  $CH_3$  and  $CH_2SO_2$ ).

**Metabolite M1.** Metabolite M1 gave a deprotonated molecular ion at  $m/z$  508.1550, which is 16 amu higher than that of parent drug and consistent with the chemical formula  $C_{26}H_{26}N_3O_6S^-$  ( $[M-H]^-$ )(calculated mass  $m/z$  508.1548, parent + O). The CID of M1 produced major fragment ions including the base peak at  $m/z$  493.1316 (loss of  $CH_3$ ), other ions at  $m/z$  476.1289 (loss of

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CH<sub>3</sub>OH), 463.1213 (loss of CH<sub>3</sub> and CH<sub>2</sub>O), 435.0897 (loss of C<sub>4</sub>H<sub>9</sub>O), 415.1543 (loss of CH<sub>3</sub> and CH<sub>2</sub>SO<sub>2</sub>). The presence of an ion at m/z 435.0897 suggests hydroxylation occurred at *tert*-butyl moiety. Comparison of HPLC retention times using co-injection analysis and characteristic MS/MS fragmentation patterns of M1 with an authentic reference standard confirmed the M1 structure to be *tert*-butyl hydroxylated form of dasabuvir.

**Metabolite M2.** The deprotonated molecular ion of M2 was observed at m/z 588.1111, which is consistent with the proposed chemical formula C<sub>26</sub>H<sub>26</sub>N<sub>3</sub>O<sub>9</sub>S<sub>2</sub><sup>-</sup> ([M-H]<sup>-</sup>) (calculated mass m/z 588.1116). The CID of M1 gave a major fragment ion at m/z 509.1267 (loss of CH<sub>3</sub>SO<sub>2</sub> group). Other fragment ions included m/z 545.1053 (loss of NCO), 494.1024 (loss of CH<sub>3</sub>SO<sub>2</sub> and CH<sub>3</sub>), 466.1202 (loss of NCO and CH<sub>3</sub>SO<sub>2</sub>), and 465.1490 (loss of NCO and SO<sub>3</sub>). The structure of M2 was proposed to be the sulfate conjugates of M1.

**Metabolite M3.** The deprotonated molecular ion of M3 was observed at m/z 684.1861, which is 192 amu higher than that of the parent drug, and consistent with the proposed chemical formula C<sub>32</sub>H<sub>34</sub>N<sub>3</sub>O<sub>12</sub>S<sup>-</sup> ([M-H]<sup>-</sup>) (calculated mass m/z 684.1869). The CID of M3 gave the main product ion at m/z 476.1284, a result of the loss of a glucuronide and CH<sub>3</sub>OH. Other product ions included m/z 641.1804 (loss of NCO), 597.1913 (loss of NCO and CO<sub>2</sub>), and 433.1223 (loss of C<sub>7</sub>H<sub>12</sub>O<sub>7</sub> and NCO). M3 was proposed to be the glucuronide conjugate of M1. M3 was produced by both *in vitro* human and rat liver microsomal incubations of M1 in the presence of uridine diphosphoglucuronic acid (UDPGA) and alamethicin (Fisher et al., 2000), confirming that M3 was formed through glucuronidation of M1. M3 isolated from rat bile was readily hydrolyzed by β-glucuronidase (Zenser et al., 1999), indicating M3 is an O-glucuronide of M1 (data not shown).



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**Metabolite M4.** The deprotonated molecular ion of M4 was observed at  $m/z$  506.1381, which is 14 amu higher than that of the parent drug and consistent with the chemical formula  $C_{26}H_{24}N_3O_6S^-$  ( $[M-H]^-$ ) (calculated mass  $m/z$  506.1391). The CID of M4 produced a major fragment ion at  $m/z$  463.1208, a result of loss of  $CH_3$  and  $CO$ . The presence of the fragment ion at  $m/z$  435.0901 (loss of  $C_4H_7O$ ) also suggested oxidation occurred on *tert*-butyl group. M4 was proposed to be the *tert*-butyl aldehyde metabolite.

**Metabolite M5.** The deprotonated molecular ion of M5 was observed at  $m/z$  522.1337, which is 30 amu higher than that of parent drug and consistent with the proposed chemical formula  $C_{26}H_{24}N_3O_7S^-$  ( $[M-H]^-$ ) (calculated mass  $m/z$  522.1340). The characteristic fragment ions included base peak  $m/z$  478.1435 (loss of  $CO_2$ ) and 463.1212 which was a result of loss of a methyl group and  $CO_2$ . The presence of ion at  $m/z$  435.0895 (loss of  $C_4H_7O_2$ ) suggested that carboxylation occurred on the *tert*-butyl group. The structure of M5 was further confirmed with an authentic synthetic reference by comparison of LC retention times and characteristic MS/MS fragmentation pattern.

**Metabolite M6.** The deprotonated molecular ion of M6 was  $m/z$  698.1657, which is consistent with the chemical formula  $C_{32}H_{32}N_3O_{13}S^-$  ( $[M-H]^-$ ) (calculated mass  $m/z$  698.1661), a glucuronide conjugate of M5 (expected mass 698.1661, molecular formula  $C_{32}H_{32}N_3O_{13}S^-$ ). The CID of M6 produced the main product ion at  $m/z$  478.1434, a result of loss of glucuronide and  $CO_2$ , and other product ions at  $m/z$  522.1334 ( $-C_6H_8O_6$ ), 463.1204 and 383.1267. M5 was also produced by both *in vitro* human and rat liver microsomal incubations of M5 in the presence of uridine diphosphoglucuronic acid (UDPGA) and the pore-forming peptide alamethicin. Based on this information, M5 was identified as the glucuronide conjugate of M5.

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**Metabolite M7.** The deprotonated molecular ion of M7 was  $m/z$  700.1801, which is consistent with the chemical formula of  $C_{32}H_{34}N_3O_{13}S^-$  ( $[M-H]^-$ ) (calculated mass  $m/z$  700.1818). The CID of ion at  $m/z$  700 yielded the fragment ion of  $m/z$  524.1493, indicating a loss of glucuronide. Further fragmentation of  $m/z$  524 by  $MS^3$  produced a fragment of  $m/z$  444.1568 (loss of  $CH_4SO_2$ ). M7 is assigned as a dihydro metabolite of M6, with addition of two protons at the pyrimidine-2,4(1H,3H)-dione moiety.

**Metabolite M8.** Metabolite M8 was only observed in feces. The deprotonated molecular ion of M8 was  $m/z$  524.1487, which is consistent with the chemical formula  $C_{26}H_{26}N_3O_7S^-$  ( $[M-H]^-$ ) (calculated mass  $m/z$  524.1497). The CID of M8 gave the main product ion at  $m/z$  465.1367, which corresponds to the loss of  $CO_2$  and a methyl group. Other product ions included  $m/z$  480.1601 (loss of  $CO_2$ ), 401.1745 (loss of  $CO_2$  and  $CH_3SO_2$ ) and 387.1593 (loss of  $CO_2$ ,  $CH_2SO_2$  and  $CH_3$ ). Based on these data, M8 was assigned as a dihydro metabolite M5, with addition of two protons at the pyrimidine-2,4(1H,3H)-dione moiety.

**Metabolite M9.** Metabolite M9 was only observed in feces. The deprotonated molecular ion of M9 was  $m/z$  510.1694 which is consistent with the chemical formula  $C_{26}H_{28}N_3O_6S^-$  ( $[M-H]^-$ ) (calculated mass  $m/z$  510.1704). The CID of M9 yielded product ions at  $m/z$  495.1474 (loss of  $CH_3$ ), 478.1445 (loss of  $CH_3OH$ ), 465.1366 (loss of  $C_2H_5O$ ), and 437.1054 (loss of  $C_4H_9O$ ). The presence of the ion at  $m/z$  437.1054 suggested a loss of tert-butyl hydroxyl. M9 was assigned as a dihydro metabolite of M1, with addition of two protons at the pyrimidine-2,4(1H,3H)-dione moiety.

**Metabolite M10.** Metabolite M10 was only observed in feces. The deprotonated molecular ion of M10 was  $m/z$  590.1260, which is consistent with the chemical formula  $C_{26}H_{28}N_3O_9S_2^-$  ( $[M-$

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H]<sup>-</sup>) (calculated mass m/z 590.1272). Due to low ion intensity, CID spectrum of M10 was not obtained. M10 was assigned as a dihydro metabolite of M2, possibly with addition of two protons at the pyrimidine-2,4(1H,3H)-dione moiety.

**Metabolite M11.** The deprotonated molecular ion of M11 was m/z 494.1380, which is consistent with the chemical formula C<sub>25</sub>H<sub>24</sub>N<sub>3</sub>O<sub>6</sub>S<sup>-</sup> ([M-H]<sup>-</sup>) (calculated mass m/z 494.1391), but is not consistent with the dihydro dasabuvir (C<sub>26</sub>H<sub>28</sub>N<sub>3</sub>O<sub>5</sub>S<sup>-</sup>, calc. mass m/z 494.1755). The observed mass has a -76 ppm mass error compared to the latter proposed structure. The CID of M11 gave product ions at 479.1155 (loss of CH<sub>3</sub>), 464.0918 (loss of two CH<sub>3</sub>), 421.0724 (loss of tert-butyl hydroxyl), 416.1612 (loss of SO<sub>2</sub>CH<sub>2</sub>), and 401.1380 (loss of SO<sub>2</sub>CH<sub>2</sub> and CH<sub>3</sub>). M11 was assigned as a desmethyl metabolite of M1.

**Unknown U1.** U1 eluted at the solvent front by HPLC and represented <5% of the radioactivity in plasma and trace amount in urine. Two potential structures are proposed for the compound (Fig. 6), each of the structures formed from the cleavage of the pyrimidine-2,4(1H,3H)-dione ring. After utilizing multiple methods to identify the compound, a definitive m/z was not obtained.

### Reaction Phenotyping of M1 formation

Identification of enzymes involved in the metabolism of dasabuvir using recombinant human CYPs and FMO enzymes *in vitro*, suggests that it is predominantly metabolized by CYP2C8, followed by CYP3A4 and to a minor extent by 2B6 and 2D6. Fig. 7 showed the representative HPLC radiochromatograms of [<sup>3</sup>H]dasabuvir and the corresponding metabolite in recombinant CYP 2C8, 3A4, 2D6 and 2B6 incubations. M1 was the only metabolite radiochemically detected in these incubations. The CYP enzyme involvement in the metabolism of dasabuvir was further

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confirmed by chemical inhibition assays. Incubation of dasabuvir in human liver microsomes in the presence of selective chemical inhibitors revealed that CYP2C8 is the major contributor to dasabuvir metabolism followed by CYP3A4 and CYP2D6 (~60, 30 and 10% of dasabuvir metabolism was inhibited by quercetin, ketoconazole and quinidine, respectively). The contribution of CYP2B6 was negligible (<1% inhibition of dasabuvir metabolism was observed with 2-phenyl-2-(1-piperidinyl)propane)).

### Discussion

The mass balance, disposition and metabolism of dasabuvir were evaluated in four healthy human male subjects. Following administration of a single 400-mg oral dose of [<sup>14</sup>C]dasabuvir, the mean total recovery of the administered radioactive dose was 96.6%, with recovery in individual subjects ranging from 90.8 to 103%. Nearly all of the administered radioactive dose (94.4%) was recovered in feces, with a limited amount of radioactivity (2.2%) recovered in urine through the last collection interval, indicating that dasabuvir and metabolites were predominantly eliminated in humans through feces and minimally through renal clearance.

LC-MS characterization of dasabuvir metabolites in urine and feces revealed that a total of eleven metabolites were present in human excreta. Biotransformation of dasabuvir in human primarily involves CYP-mediated hydroxylation at the *tert*-butyl group to form the active metabolite M1 (approximately similar activity against genotype 1 HCV infection as dasabuvir), which is also produced in preclinical toxicology rodent species with adequate safety coverage (Abbvie internal data). M1 is further oxidized to M4 (*tert*-butyl aldehyde) and M5 (*tert*-butyl acid). Subsequent glucuronidation of M1 and M5 produces metabolite M3 (*tert*-butyl ether glucuronide) and M6 (*tert*-butyl acid glucuronide), respectively; M2 is derived from the

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sulfation of M1. Reduction of M5, M1, and M2 at the pyrimidine-2,4(1H,3H)-dione moiety leads to formation of M8, M9 and M10, respectively. Metabolites M8, M9 and M10 were only observed in human feces. Trace levels of M7 (a reduction product of M6) and M11 (O-desmethyl metabolite of M1) were also detected in human urine.

Of the total radioactivity excreted in human feces and urine, unchanged parent drug constituted 26.2% of total dose, and dasabuvir metabolites accounted for approximately 70% of the dose, suggesting the absorption of [<sup>14</sup>C]dasabuvir in human was greater than 70% of the dose. M1 was the most abundant radiochemical component in human feces, accounting for 31.5% of total dose, followed by M2 (15.2%) and M5 (11.1%). The total amount of M1-related conjugates in human urine and feces accounted for 23.9% of dose. Secondary oxidative metabolites of M1 and subsequent conjugates comprised 13.8% of dose, indicating that M1 is mainly cleared through fecal elimination as intact or conjugates; subsequent oxidation of M1 plays a secondary role.

Unchanged dasabuvir was the major component of total drug related radioactivity in plasma. Seven metabolites were identified in plasma, including the *tert*-butyl hydroxylate M1 and minor metabolites M2, M3, M4, M5, M6, M11. The principal component in plasma was unchanged parent drug with an AUC<sub>0-∞</sub> of 6290 ng•hr/mL. The AUC<sub>0-∞</sub> of the most abundant plasma metabolite M1 was 2280 ng•hr/mL. Metabolite profiling indicated that dasabuvir represented approximately 58% of the total radioactivity and M1 accounted for about 21% that of total drug-related materials. Other metabolites, including M2, M3, M4, M5, M6, and M11, were relatively minor, ranging from trace levels up to 6.6% of total drug in plasma.

Metabolism involving *tert*-butyl oxidation to form M1 is one of the primary clearance pathways for elimination of dasabuvir in human. CYP enzyme characterization of this pathway is essential

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to understand clinical drug-drug interactions. The *in vitro* P450 reaction phenotyping results indicated the importance of CYP2C8 followed by CYP3A4 in the dasabuvir metabolism, with quercetin inhibiting approximately 60% of dasabuvir hepatic metabolism *in vitro*. These results are consistent with the clinical findings (Menon et al., 2014) showing that CYP2C8 inhibition increased dasabuvir exposure (2-fold  $C_{\max}$ , 11-fold  $AUC_{0-72h}$  increase) after co-administration with gemfibrozil, while the M1 metabolite  $C_{\max}$  decreased by 20-fold and  $AUC_{0-72}$  decreased by 4-fold after administration of dasabuvir alone, indicating that CYP2C8 plays an important role in dasabuvir metabolism in humans. M1 is mainly cleared through direct biliary/fecal elimination and through further oxidation to M5 (tert-butyl acid) or glucuronidation to M2 (t-butyl hydroxyl glucuronide). Recombinant CYP phenotyping study indicated M1 is predominantly metabolized by CYP3A4 with no metabolism observed by other CYPs. M5 is an inactive metabolite, present at low levels in circulation. Detailed characterization of *in vitro* CYP 450 and transporter assays and physiologically based pharmacokinetic models to provide mechanistic understanding of potential DDIs involving dasabuvir, M1 metabolite, and other DAA components were reported elsewhere (Shebley *et al.*, 2016).

In summary, the overall disposition and metabolism of dasabuvir has been determined in healthy volunteers. Dasabuvir is well absorbed and extensively metabolized through *tert*-butyl hydroxylation to metabolite M1, followed by glucuronidation or sulfation of M1 or subsequent secondary oxidation pathways. Dasabuvir is eliminated by the biliary-fecal route. Renal excretion of dasabuvir and metabolites is deemed to be negligible. All the metabolites identified in this study were also present in the preclinical safety species.

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**Authorship Contributions**

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

Conducted experiments: Serby, Zhang, Wan.

Contributed new reagents or analytic tools: Serby, Reed.

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

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

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Retreatment of HCV with ABT-450/r-ombitasvir and dasabuvir with ribavirin. *N Engl J Med*  
370:1604-1614.

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**Unnumbered Footnote to the Title**

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### Figure Legends

Fig. 1. Structure of [<sup>14</sup>C]dasabuvir and synthetic metabolites

Fig. 2. Mean cumulative percent of radioactive dose recovered in urine and feces at specified intervals after a single 400-mg (100- $\mu$ Ci) oral dose of [<sup>14</sup>C]dasabuvir to healthy male subjects

Fig. 3. Mean (standard deviation) plasma concentration-time curves for dasabuvir (ng/mL) and total radioactivity (ng-eq/g) in male subjects administered a single oral dose of [<sup>14</sup>C]dasabuvir 400 mg (n=4).

Fig. 4. Representative HPLC radiochromatograms of dasabuvir and its metabolites in human plasma after a single 400-mg oral dose of [<sup>14</sup>C]dasabuvir.

Fig. 5. Representative HPLC radiochromatograms of dasabuvir and its metabolites in human excreta, (A) urine and (B) feces, after a single 400-mg oral dose of [<sup>14</sup>C]dasabuvir.

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

Fig. 7. Representative HPLC radiochromatograms of dasabuvir and its metabolites in recombinant human CYP2C8 (a), CYP3A4 (b), CYP2D6 (c) and CYP2B6 (d) isoforms.

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**Tables**

Table 1. Mean  $\pm$  SD pharmacokinetic parameters of total radioactivity, dasabuvir and metabolite M1

<b>Analyte</b>	<b>C<sub>max</sub></b> (ng-eq/g or ng/mL)	<b>T<sub>max</sub></b> (h)	<b>AUC<sub>0-last</sub></b> (ng-eq•h/g or ng•h/mL)	<b>AUC<sub>0-∞</sub></b> (ng-eq•hr/g or ng•h/mL)	<b>T<sub>1/2</sub></b> (h)
Total Radioactivity	1300 $\pm$ 586	4 $\pm$ 0	8251 $\pm$ 3111	9959 $\pm$ 3414	
Dasabuvir	658 $\pm$ 252	4 $\pm$ 0	6260 $\pm$ 1930	6290 $\pm$ 1930	8.4 $\pm$ 3.2
M1	267 $\pm$ 116	3.0 $\pm$ 1.2	2230 $\pm$ 948	2280 $\pm$ 934	6.2 $\pm$ 1.3

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Table 2. Percentages (mean  $\pm$  SD) of radioactivity for dasabuvir and corresponding metabolites in human plasma following administration of a single 400-mg oral dose of [ $^{14}\text{C}$ ]dasabuvir (n=4)

	<b>Percentage of Radioactivity in Plasma (<math>\pm</math> SD)</b> (n=4)
<b>Dasabuvir</b>	58.1 $\pm$ 4.6
<b>M1</b>	21.4 $\pm$ 2.4
<b>M2</b>	6.6 $\pm$ 2.4
<b>M3</b>	4.4 $\pm$ 3.0
<b>M4</b>	1.7 $\pm$ 1.3
<b>M5</b>	1.7 $\pm$ 1.7
<b>M6</b>	2.3 $\pm$ 2.0
<b>U1</b>	4.7 $\pm$ 1.1

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Table 3. Percentages of excretory metabolites of dasabuvir in humans following administration of a single 400-mg oral dose of [<sup>14</sup>C]dasabuvir (n=4)

<b>Metabolite</b>	<b>Feces 0-216 h</b>	<b>Urine 0-24 h</b>	<b>Total</b>
<b>Total Radioactivity</b>	94.4	2.20*	96.6
<b>Dasabuvir</b>	26.2	0.03	26.2
<b>M1</b>	31.5	0.85	32.4
<b>M2</b>	15.2	0.3	15.5
<b>M3</b>	ND	0.19	0.19
<b>M4</b>	ND	0.12	0.12
<b>M5</b>	11.1	0.12	11.2
<b>M6</b>	ND	0.02	0.02
<b>M7</b>	ND	0.38	0.38
<b>M8</b>	2.05	ND	2.05
<b>M9</b>	4.91	ND	4.91
<b>M10</b>	3.37	ND	3.37
<b>M11</b>	ND	0.03	0.03
<b>U1</b>	ND	0.01	0.01

\* Total radioactivity for urine is 0 – 216 h

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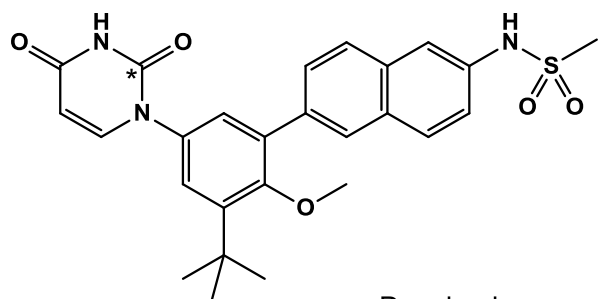
Table 4. Retention time, molecular ion and characteristic fragment ions of dasabuvir and metabolites in human plasma, urine or feces

Compound	Observed [M-H] <sup>-</sup>	Theoretical [M-H] <sup>-</sup>	Δ ppm	Metabolite ID	Characteristic Fragment Ions
Dasabuvir	492.1601	492.1599	0.41	Parent drug	<u>477</u> (-CH <sub>3</sub> ), 462(-2 CH <sub>3</sub> ), 449(-NCO), 435 (-C <sub>4</sub> H <sub>9</sub> ), 414 (- SO <sub>2</sub> CH <sub>2</sub> ), 399 (- SO <sub>2</sub> CH <sub>2</sub> - CH <sub>3</sub> )
M1	508.1550	508.1548	0.39	P +O	<u>493</u> (-CH <sub>3</sub> ), 476(-CH <sub>3</sub> OH), 463(- C <sub>2</sub> H <sub>5</sub> O), 435(-C <sub>4</sub> H <sub>9</sub> O), 430(- SO <sub>2</sub> CH <sub>2</sub> ), 415(- SO <sub>2</sub> CH <sub>2</sub> - CH <sub>3</sub> ), 399 (- SO <sub>2</sub> CH <sub>2</sub> - CH <sub>3</sub> O)
M2	588.1111	588.1116	-0.85	M1 sulfate	545 (-NCO) <u>509</u> (-CH <sub>3</sub> SO <sub>2</sub> ), 494(- CH <sub>3</sub> SO <sub>2</sub> -CH <sub>3</sub> ), 466(-NCO- CH <sub>3</sub> SO <sub>2</sub> ), 465 (-NCO-SO <sub>3</sub> ), 433 (-NCO-CH <sub>4</sub> SO <sub>4</sub> )
M3	684.1861	684.1869	-1.17	M1 glucuronide	641(-NCO), 597(-NCO-CO <sub>2</sub> ), <u>476</u> (- C <sub>7</sub> H <sub>12</sub> O <sub>7</sub> ), 433 (-C <sub>7</sub> H <sub>12</sub> O <sub>7</sub> -NCO)
M4	506.1381	506.1391	-1.98	M1 -2H	<u>463</u> (-C <sub>2</sub> H <sub>3</sub> O), 435 (-C <sub>4</sub> H <sub>7</sub> O), 413 (- SO <sub>2</sub> CH <sub>2</sub> - CH <sub>3</sub> )
M5	522.1337	522.1340	-0.57	P+2O-2H	507(-CH <sub>3</sub> ), 478 (-CO <sub>2</sub> ), <u>463</u> (-C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ), 435 (-C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> )
M6	698.1657	698.1661	-0.57	M5 glucuronide	522 (-C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> ), <u>478</u> (-C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> - CO <sub>2</sub> ), 463(-C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> - CO <sub>2</sub> - CH <sub>3</sub> ), 383 (- C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> - CO <sub>2</sub> - CH <sub>3</sub> - CH <sub>4</sub> SO <sub>2</sub> )
M7	700.1801	700.1818	-2.43	M6 + 2H	<u>524</u> (-C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> ), 444 (-C <sub>6</sub> H <sub>8</sub> O <sub>6</sub> - CH <sub>4</sub> SO <sub>2</sub> )
M8 <sup>a</sup>	524.1487	524.1497	-1.91	M5 + 2H	480(-CO <sub>2</sub> ), <u>465</u> (-C <sub>2</sub> H <sub>3</sub> O <sub>2</sub> ), 437(- C <sub>4</sub> H <sub>7</sub> O <sub>2</sub> ), 401 (-CO <sub>2</sub> -CH <sub>3</sub> SO <sub>2</sub> ), 387(- CO <sub>2</sub> -CH <sub>2</sub> SO <sub>2</sub> -CH <sub>3</sub> )
M9 <sup>a</sup>	510.1694	510.1704	-1.96	M1 + 2H	<u>495</u> (-CH <sub>3</sub> ), 478 (-CH <sub>3</sub> OH), 465 (- C <sub>2</sub> H <sub>5</sub> O), 452,437(-C <sub>4</sub> H <sub>9</sub> O)
M10 <sup>a</sup>	590.1260	590.1272	-2.03	M2 + 2H	N/A
M11	494.1380	494.1391	-2.33	M1 - CH <sub>2</sub>	<u>479</u> (-CH <sub>3</sub> ), 464(-2 CH <sub>3</sub> ), 416(- SO <sub>2</sub> CH <sub>2</sub> ), 401(- SO <sub>2</sub> CH <sub>2</sub> - CH <sub>3</sub> )

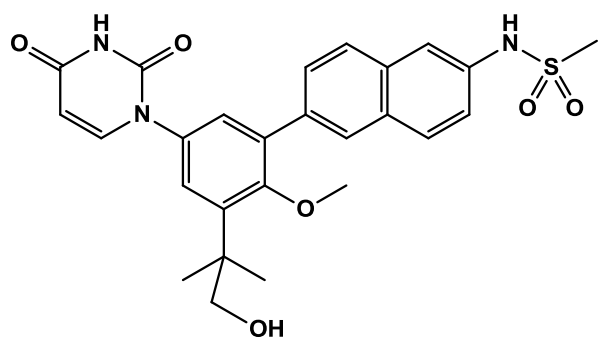
a Observed only in fecal samples.



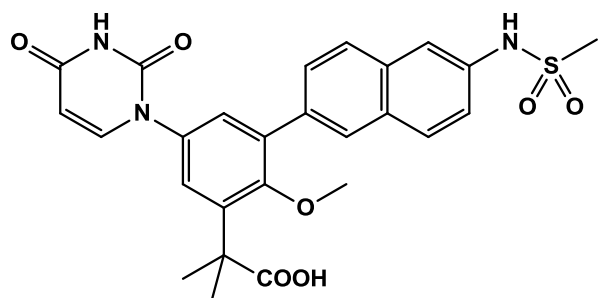
Figure 1.



Dasabuvir  
\* Position of radiolabel



M1



M5

Fig. 2.

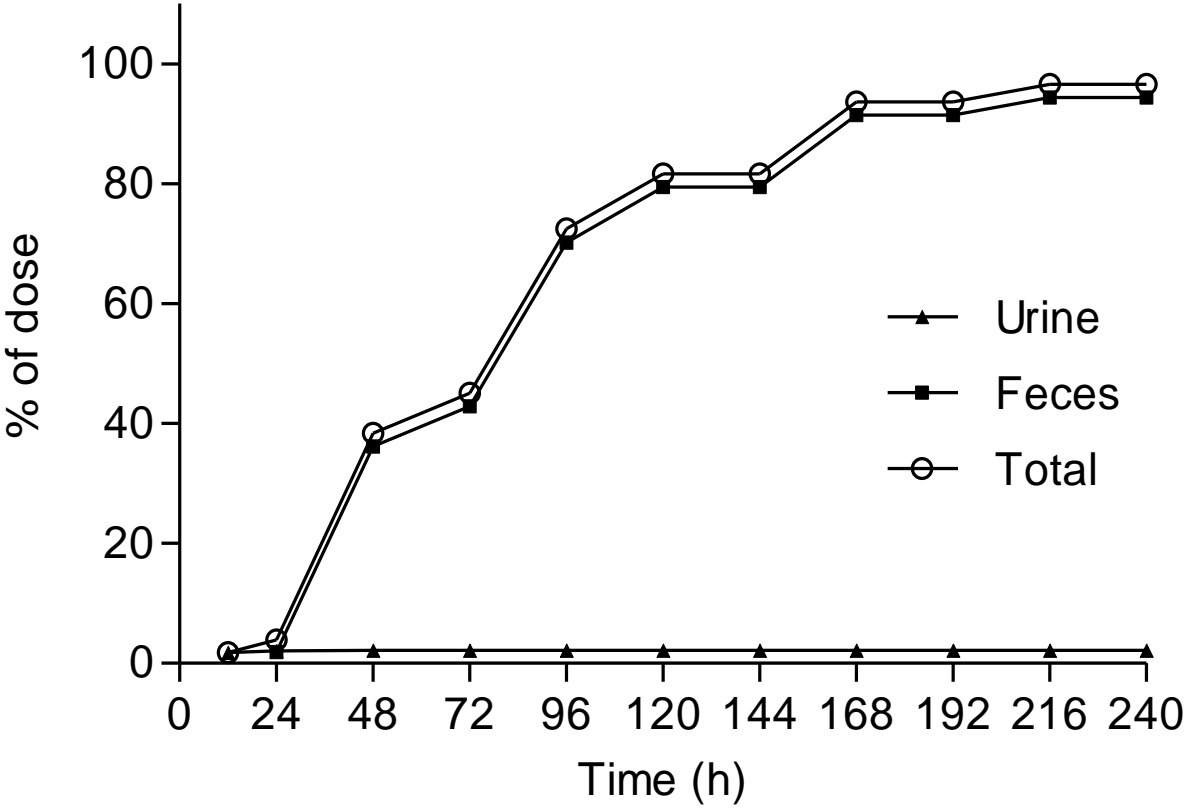


Fig. 3.

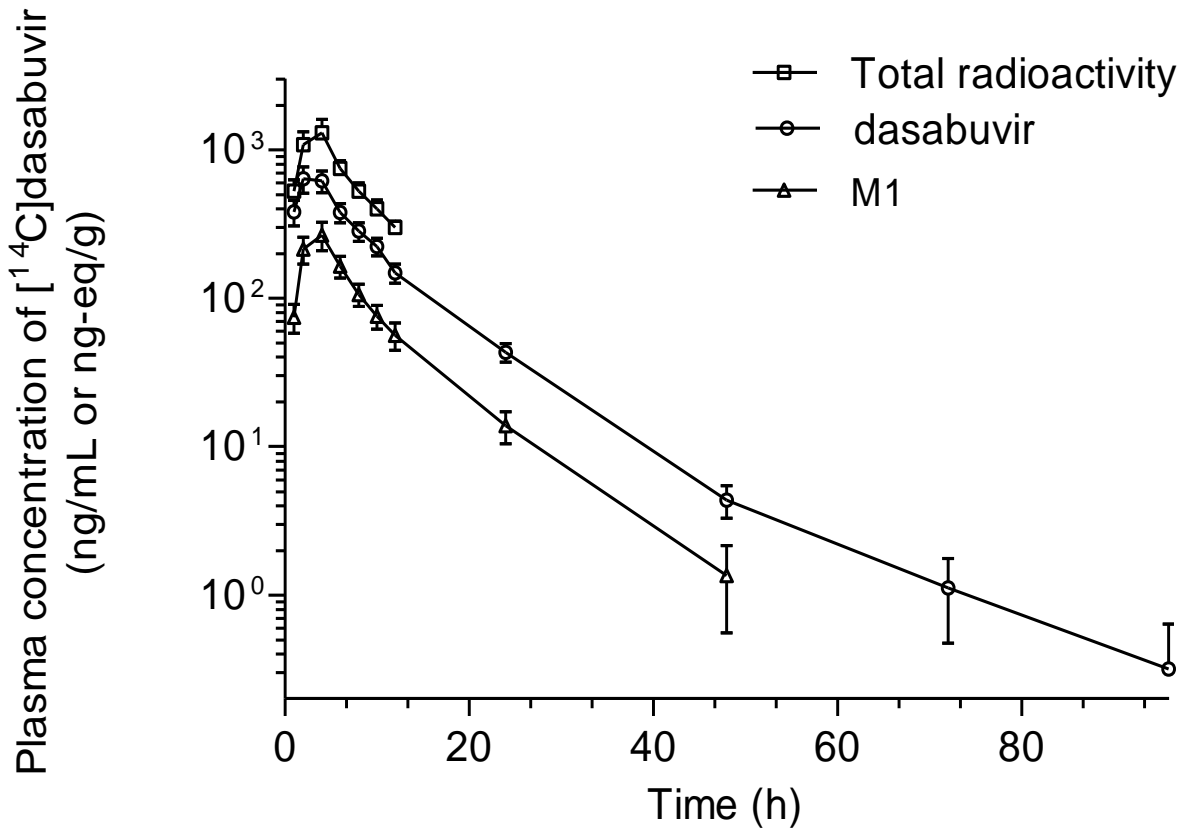


Fig. 4.

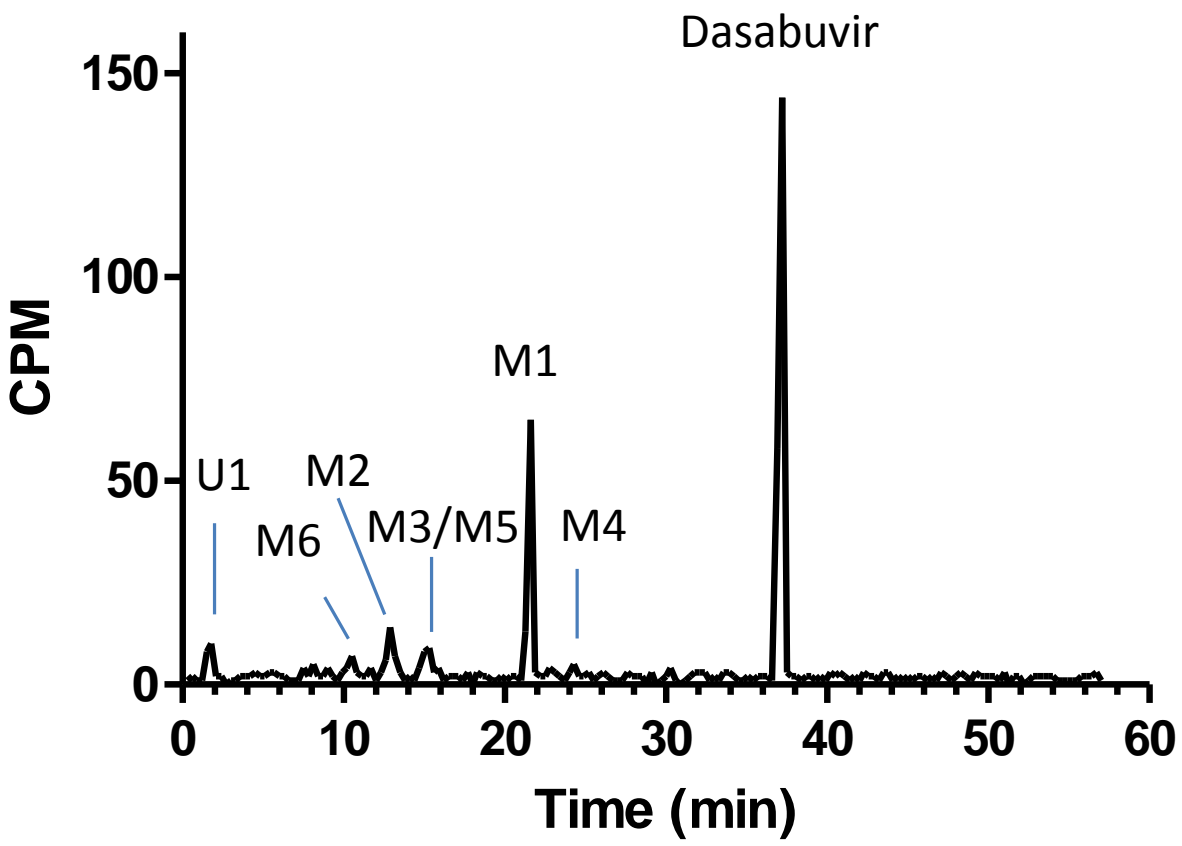


Fig. 5.

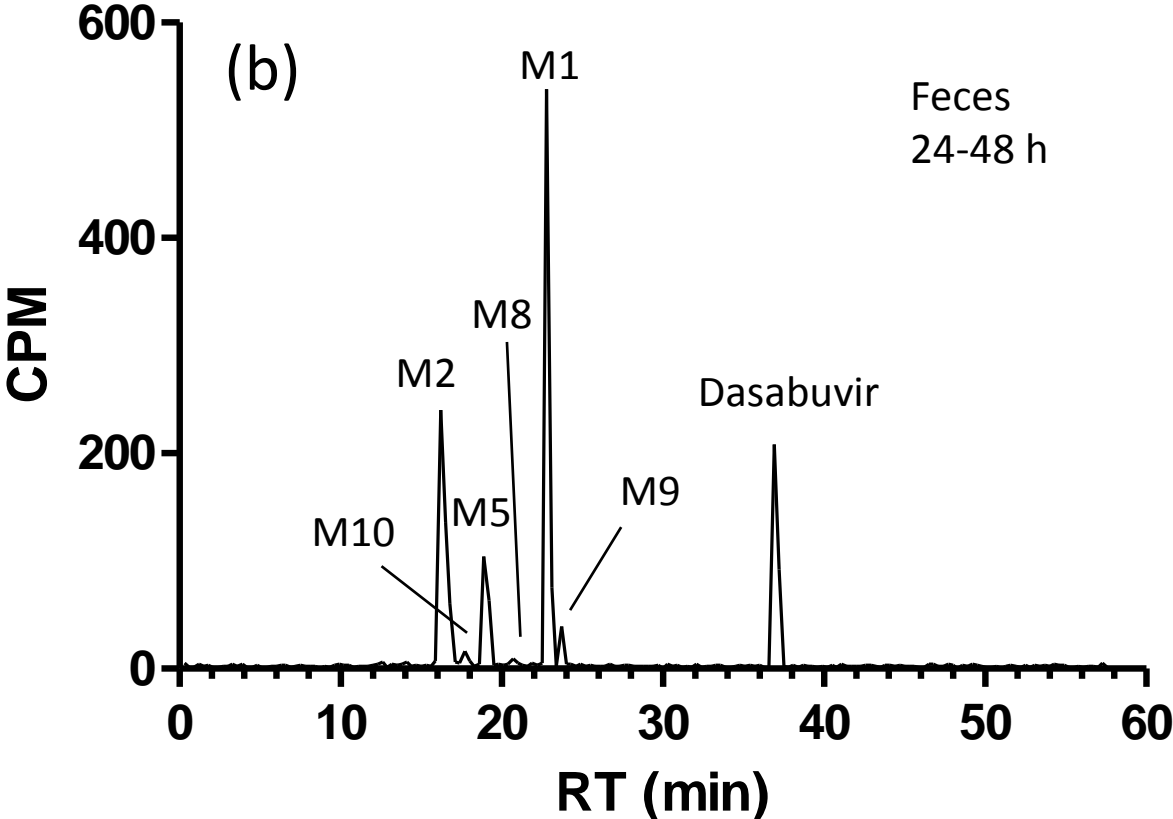
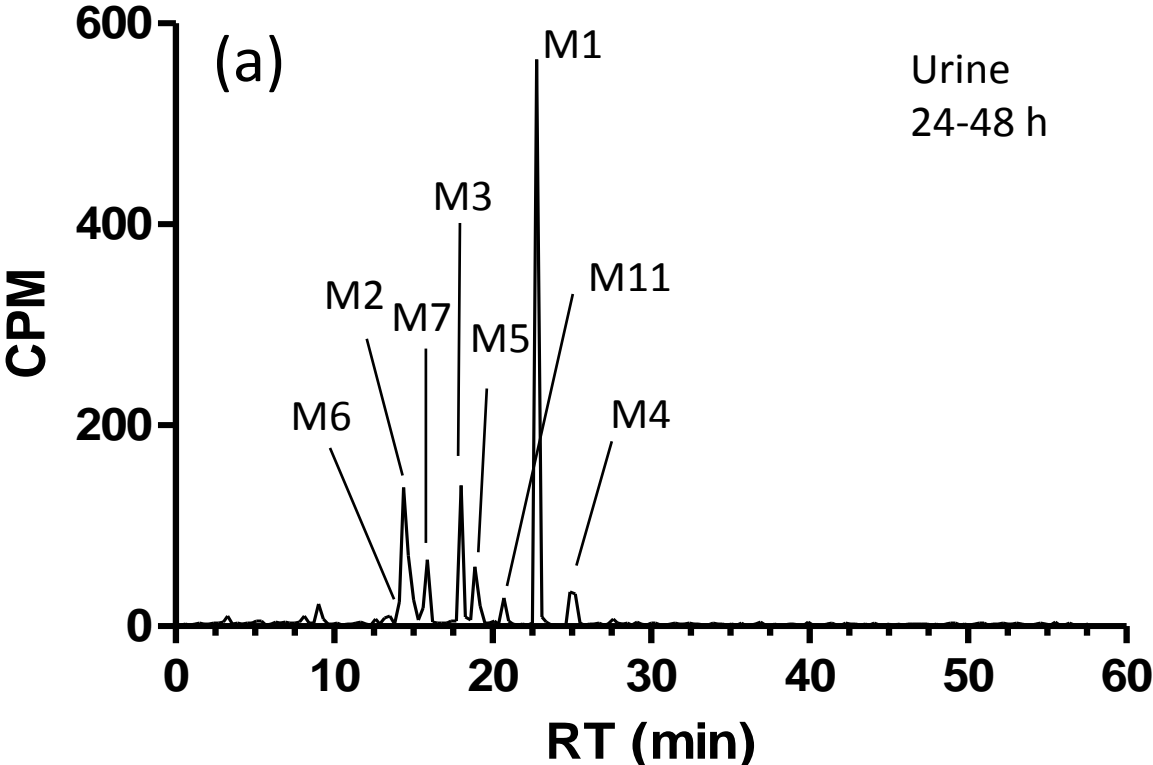


Fig. 6.

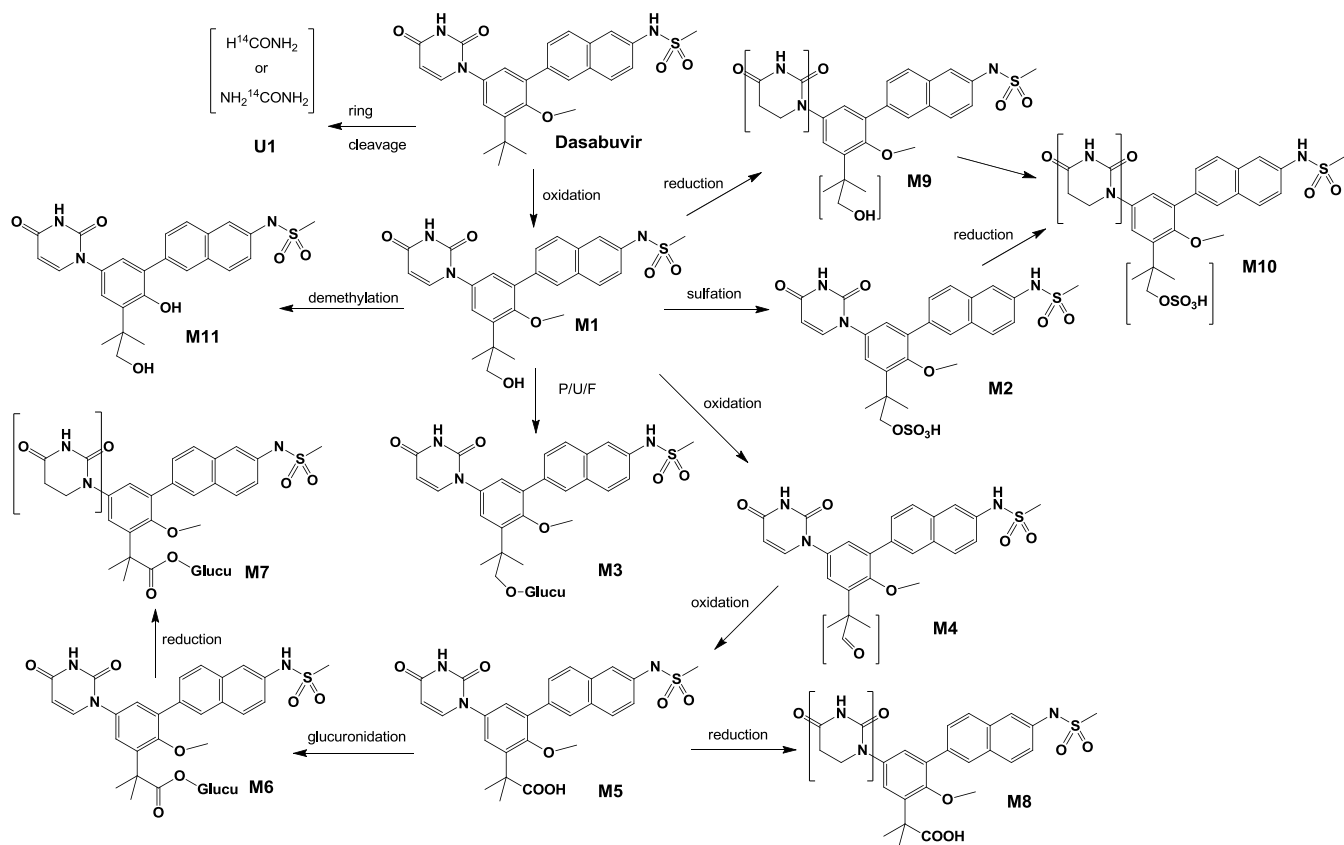


Fig. 7.

