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Determination of simeprevir: A novel, hepatitis C protease inhibitor in human plasma by high-performance liquid chromatography–tandem mass spectrometry

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ABSTRACT

Simeprevir (also known as TMC435 or TMC435350) is a novel hepatitis C protease inhibitor. A validated high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the sensitive and selective quantification of simeprevir in human EDTA plasma is described. During assay development, special attention was given to light instability of the drug in plasma and blood. The method consisted of precipitation of plasma proteins with acetonitrile after which the supernatant was analyzed using electrospray LC–MS/MS. The linearity was confirmed in the concentration range from 2.00 to 2000 ng/mL, with 50-fold dilution extending to 100,000 ng/mL. The precision of this assay, expressed as CV, ranged between 4.4% and 8.5% over the entire concentration range with assay accuracy between −0.3% and 8.5%. The method was applied successfully in many clinical studies to document the pharmacokinetics of simeprevir in plasma from healthy volunteers and patients.

1. Introduction

Hepatitis C is one of the most common viral liver diseases (after hepatitis B) and is caused by the hepatitis C virus (HCV). It is estimated that 170 million patients worldwide and about 2% of the population in developed countries and up to 9% in developing countries are chronically infected with HCV [1]. The majority of acute HCV infections become chronic, some of which progress towards liver cirrhosis or hepatocellular carcinoma [2–6]. However, HCV is an asymptomatic, slowly progressive disease evolving over 10–20 years. In some cases, hepatitis C can remain asymptomatic even after significant liver damage has occurred.

Since the discovery of the virus, there have been many advances in hepatitis C research. The current standard of care is pegylated interferon alpha 2a and -alpha 2b in combination with ribavirin, which has a sustained viral response rate of 40–50% in genotype 1 HCV-infected patients, which accounts for the majority of the hepatitis C population in the United States and Japan, and of 80–90% in patients infected with genotype 2 or 3 HCV [7–12]. Moreover, this therapy may be poorly tolerated and can cause serious adverse effects during the 48-weeks treatment. This means that more effective therapeutic drugs with fewer side effects such as skin problems, weight loss, depression, anaemia, neutropenia, and thrombocytopenia and shorter treatment durations are needed for patients infected with HCV.

Simeprevir is a novel, oral, selective, highly potent, HCV NS3/4A-protease inhibitor. Its efficacy and safety have been demonstrated in clinical phase I–II–III trials. In order to assess the human pharmacokinetics of simeprevir, a validated, robust and selective bioanalytical assay was required allowing the quantification of simeprevir; both when dosed alone or in combination with the standard of care (pegylated interferon α in combination with ribavirin).

The assay described was validated according to current Guidelines on bioanalytical method validation [13,14] and considering the proceedings from the Crystal City III conference report [15]. Initially, a bioanalytical assay was developed and validated using liquid–liquid extraction as sample clean-up. In order to increase the bioanalytical throughput and to facilitate sample clean up when analysing thousands of samples from phase II and phase III studies, the sample preparation was modified to protein precipitation.

2. Experimental

2.1. Chemicals

Simeprevir (2*R*,3a*R*,10*Z*,11a*S*,12a*R*,14a*R*)-*N*-(cyclopropylsulfonyl)-2-[[2-(4-isopropyl-1,3-thiazol-2-yl)-7-methoxy-8-methyl-4-quinolinyl]oxy]-5-methyl-4,14-dioxo-2,3,3a,4,5,6,7,8,9,11a,12,13,14,14a-tetradecahydrocylopenta[*c*]cyclopropa[*g*][1,6]diazacyclotetradecine-12a-(1*H*)-carboxamide ($C_{38}H_{47}N_5O_7S_2$, MW 749.94,

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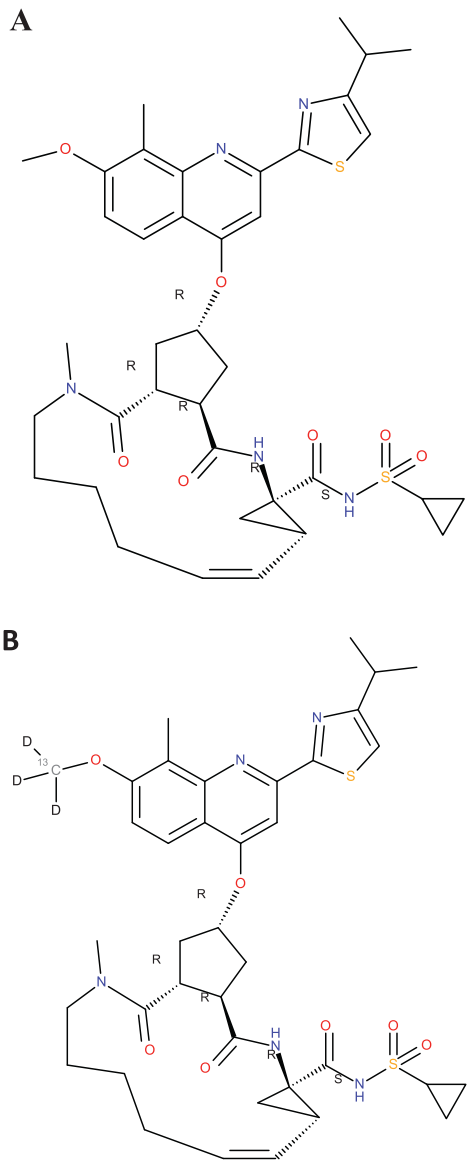

Fig. 1. Chemical structure of (A) simeprevir and (B) STIL internal standard.

Fig. 1A) is a single enantiomer containing 5 asymetric carbon atoms, a double bond and a 14-membered macrocycle, shown in Fig. 1A; the STtable Isotope Labelled Internal Standard (STIL-IS) ($^{13}C_1C_{37}H_{44}D_3N_5O_7S_2$) is shown in Fig. 1B. Both were obtained from Janssen Research and Development (Beerse, Belgium). Ammonium formate and formic acid were of analytical grade and were obtained from Merck (Darmstadt, Germany). Methanol and acetonitrile were of spectrophotometric grade. Methanol was obtained from Merck (Darmstadt, Germany) and acetonitrile was from Sigma–Aldrich. Water was purified by the Milli Q system (Millipore). Blank human EDTA plasma was obtained from Bioreclamation (New York, USA).

2.2. Instrumentation

An Applied Biosystems (AB) Sciex API-4000 tandem mass spectrometer equipped with a TurboIonspray™ interface, a Shimadzu SIL-HTc autosampler and an Agilent/HP-1100 system were used for the LC–MS/MS analyses.

Peak area integrations were carried out using the validated PC software Analyst (Applied Biosystems) version 1.4.1, 1.4.2 and 1.5.1 starting from method development till analysis of study samples in PhIII studies.

Calculations were done using Watson LIMS (Thermo Electron Corporation) version 7.2 or 7.3.

Calibration curves were created by plotting the log-transformed peak area ratios against the nominal log-transformed simeprevir standard concentrations. Final concentrations were obtained by back-calculation from the calibration curves.

Table 1
Gradient elution conditions for determination of simeprevir concentrations in human plasma, with A = ammonium formate 0.01 M pH 4 with formic acid, B = acetonitrile and C = methanol.

Time (min)	Flow (mL/min)	%A	%B	%C
0.00	1.2	25	65	10
2.00	1.2	25	65	10
2.10	1.7	1	1	98
2.90	1.7	1	1	98
2.91	1.2	25	65	10
4.00	1.2	25	65	10

2.3. Assay history

Originally, the assay consisted of a liquid–liquid extraction, chromatographic separation on a RP-18 stationary phase, followed by MS/MS detection. For the reasons mentioned above, liquid–liquid extraction was replaced by protein precipitation. Method robustness was further improved by preparing calibration standards in bulk that were stored frozen until use (instead of daily spiking calibration samples from standard solutions), and by changing HPLC conditions. Cross validation of the assays was done using independent QCs. All validated assays complied with the acceptance criteria applicable for regulated bioanalysis. Procedural details of the final assay are given in following paragraphs.

2.4. Assay description

A 100 μL aliquot of the samples was transferred to a 96-well round deep-well microplate (Porvair) and spiked with 100 μL methanol and 50 μL STIL-IS (200 ng/ml methanol). Proteins were then precipitated with 350 μL acetonitrile, followed by vigorous mixing for 3 min using a VX-2500 Multitube Vortexer and centrifugation for 10 min at 5000–6000 g. Two μL from the supernatant was injected on the LC–MS/MS system.

Chromatographic separation was achieved using a gradient mobile phase on a reversed phase 4.6 mm × 30 mm column packed with 3.5 μm C18-Xbridge (Waters) operating at 40 °C. Details are given in Table 1.

Detection was done by a Triple Quadrupole Mass Spectrometer API-4000 with a TurboIonspray™ interface. The TurboIonspray™ ionsource was set at 500 °C with an ionization voltage of 5 kV. The nebulizing gas (N_2) and curtain gas flows (N_2) were set at 50 and 40 arbitrary units, respectively, and the declustering potential at 40 V. Collision-induced fragmentation at Q2 occurred at a collision energy of 47 eV. The dwell time was 300 ms and mass analysers Q1 and Q3 were operated at unit mass resolution. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode (m/z 750.3 → 315.0 for simeprevir and at m/z 754.3 → 319.0 for the STIL-IS).

The compound eluted at a retention time of approximately 2.11 min and showed to be selective towards circulating metabolites. In Fig. 2, a product ion scan of simeprevir, acquired in positive ion mode by infusing the standard solution at a concentration of 20 μg/mL, and a chromatogram of simeprevir and its STIL IS of a real patient sample, taken at 6 h post-dose are shown.

The LC–MS/MS assay for determination of simeprevir in human EDTA plasma was transferred to 2 different contract labs and was used to analyze samples originating from phase I, phase II and