Development and validation of a UPLC–MS/MS method for the simultaneous determination of paritaprevir and ritonavir in rat liver

Aim: Determination of paritaprevir and ritonavir in rat liver tissue samples. **Results:** We successfully validated a UPLC–MS/MS method to measure paritaprevir and ritonavir in rat liver using deuterated internal standards (*d8*-paritaprevir and *d6*-ritonavir). The method is linear from 20 to 20,000 and 5 to 10,000 pg on column for paritaprevir and ritonavir, respectively, and is normalized per milligram tissue. Interday and intraday variability ranged from 0.591 to 5.33% and accuracy ranged from -6.68 to 10.1% for quality control samples. The method was then applied to the measurement of paritaprevir and ritonavir in rat liver tissue samples from a pilot study. **Conclusion:** The validated method is suitable for measurement of paritaprevir and ritonavir within rat liver tissue samples for PK studies.

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• UPLC–MS/MS • validation

The global impact of hepatitis C virus (HCV) infection is reflected in approximately 185 million individuals worldwide with an estimated mortality of 350,000 per year [1]. In the USA, approximately 5 million individuals have chronic HCV infection, with a majority of these cases occurring in people born between 1945 and 1965 [2–4]. Patients with chronic HCV infection can progress to hepatic cirrhosis, end-stage liver disease and hepatocellular carcinoma.

Paritaprevir (PTV) is a direct-acting antiviral (DAA) used in the treatment of HCV [5]. PTV is an HCV NS3/4A protease inhibitor approved by the US FDA for use in combination with ombitasvir (NS5A inhibitor) and dasabuvir (non-nucleoside NS5B polymerase inhibitor) with or without ribavirin for the treatment of HCV genotype 1 infection [6,7]. PTV is a CYP450 3A substrate, which requires PK enhancement with ritonavir (RTV) at a dose of 100 mg given concurrently [8]. PTV obtains a PK profile

that allows for once daily dosing when used in combination with RTV.

To support evolving research into the clinical PK assessment of PTV, information on concentrations of the drug in liver tissue is of great importance because the liver is the site of action for HCV DAAs. Since PTV utilizes the pharmacoenhancement properties of RTV to improve its PK profile, it is essential to have an assay for both drugs in order to gain an understanding of their interplay within the liver. For human liver tissue, sample collection methods include core needle biopsy (CNB) and fine needle aspirate (FNA). While both collection methods are invasive, the latter has been found to be associated with less risk of morbidity [9–13]. The equivalence of drug concentrations between these two sample types and the actual liver tissue itself is unknown and therefore a UPLC–MS/MS method to measure PTV and RTV in liver is important.

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Several assays have been published that describe the measurement of RTV by use of chromatography with ultraviolet detection [14,15] or by MS detection [16–23]. The measurement of RTV by high performance LC with UV detection from mouse liver samples has been described previously [24]. However, the assay utilized for this method involved dilution of a mouse liver homogenate into calf serum and quantitation was based on a set of serum matrix calibrators. Ideally, a method should have calibrators set up in the same matrix as unknown samples. To the best of our knowledge, there are no published methods for measurement of paritaprevir in rat liver tissue. Here we describe a novel UPLC–MS/MS method developed for simultaneous measurement of paritaprevir and ritonavir in rat liver tissue and its application to a rat dosed with PTV and RTV.

Experimental

Chemicals & reagents

Paritaprevir, *d*8-paritaprevir and ritonavir, were obtained from AbbVie (North Chicago, IL, USA). *d*6-Ritonavir was purchased from Toronto Research Chemicals (Toronto, Ontario, CA). LC–MS solvents included acetonitrile and methanol obtained from Acros Organics through Fisher Scientific (Pittsburgh, PA, USA). OmniSolv® grade water was obtained from Millipore (Billerica, MA, USA). Ultra-pure grade (>99.9%) nitrogen gas and argon gas were obtained from Jackson Welding and Gas Products (Buffalo, NY, USA).

UPLC–MS/MS

LC was performed with a Waters ACQUITY UPLC® H-Class System (Milford, MA, USA), which included an autosampler, an ultra-high performance quaternary pump and a column heater. Chromatographic separation was achieved with a Waters ACQUITY BEH C18 (2.1 × 50 mm, 1.7 µm) analytical column, connected to a VanGuard C18 (2.1 × 5 mm, 1.7 µm) precolumn held at 45°C in a column heater. The flow rate was 300 µl/min and composed of solvent A (95:5:0.1, water:acetonitrile:formic acid v/v/v), and solvent B (acetonitrile containing 0.1% formic acid). The gradient comprised 40% solvent A and 60% B for 0–0.5 min, followed by a linear gradient to 85% B from 0.5 to 1.0 min, then to 95% B from 1.0 to 1.1 min, held at 95% B for 0.9 min then set to initial conditions from 2.01 to 4.25 min. The total run time was 4.25 min. The autosampler was maintained at 10°C.

MS/MS detection was performed on a Waters ACQUITY TQD triple quadrupole mass spectrometer (Milford, MA, USA) equipped with an electrospray ionization source in positive ion mode (ESI+). The

spray voltage was set to 3200V with desolvation gas set at 800 l/h. The vaporizer temperature and source temperature were set to 400 and 150°C, respectively. Collision gas (argon) pressure was set at 1.5 mTorr. The ion transitions were *m/z* 766 → 571 for PTV (collision energy = 20V), *m/z* 774 → 571 for *d*8-PTV (collision energy = 22V), *m/z* 721 → 140 for RTV (collision energy = 78V) and *m/z* 727 → 146 for *d*6-RTV (collision energy = 66V). Signal output was captured and processed with Empower™ 3 software (Waters, Milford, MA, USA).

Preparation of calibration standards & QC samples

Each analyte (PTV, RTV and internal standards) was dissolved to obtain a 1.0 mg/ml stock solution; PTV was dissolved in acetonitrile and RTV was dissolved in methanol. Stock solutions were then mixed with 50:50 acetonitrile:water to prepare intermediate stock solutions from which acetonitrile-based calibration standards were prepared at 10× final concentration. These 10× acetonitrile-based standards were diluted 1:10 into blank liver homogenate for each chromatographic run to create standards at concentrations of 20.0, 50.0, 200, 1000, 5000, 10,000, 16,000 and 20,000 pg on column (5.00–5000 ng/ml) for PTV and 5.00, 10.0, 100, 500, 2500, 5000, 8000 and 10,000 pg on column (1.25–2500 ng/ml) for RTV. The blank liver homogenate was prepared by homogenizing approximately 10 mg rat liver tissue in 400 µl acetonitrile with the Next Advance Bullet Blender® Storm (Averill Park, NY, USA) in the same fashion as study samples described in the sample preparation section below. Three quality control samples (LQC, MQC and HQC) were made by spiking with appropriate volumes of separate 1.0 mg/ml stock solutions into blank liver tissue homogenate supernatant to achieve concentrations of 60.0, 4000 and 15,000 pg on column for PTV and 15.0, 2000, 7500 pg on column for RTV. All stock solutions and standards were stored at -70°C. QC samples were also stored at -70°C to simulate the storage conditions of study samples.

Sample preparation

Liver tissue samples (5–35 mg wet weight) were homogenized with a Next Advance Storm Bullet Blender® at ambient temperature. Tissue was added to a 1.5 ml Eppendorf® Safe-lock™ tube, the weight was recorded and 400 µl of acetonitrile was added along with 100 µl (1 scoop) of 0.5 mm-diameter zirconium oxide beads. Tissue was homogenized with the Bullet Blender® Storm at setting 12 for 5 min. The samples were centrifuged post homogenization at 10,000 × *g* for 5 min at ambient temperature. Clear supernatant was trans-

ferred off from the spent liver tissue and bead mixture. Samples were processed for UPLC–MS/MS analysis by adding 25 µl internal standard (150 ng/ml *d8*-PTV and *d6*-RTV) to 100 µl of centrifuged homogenate supernatant and last diluted with the addition of 125 µl of mobile phase A. Ten microliters were injected onto the separation system.

Assay validation

Calibration & linearity

Calibration curves were constructed using eight concentrations of each analyte. For each curve, the absolute peak-area ratios of the analyte to their corresponding stable isotope-labeled internal standard were calculated and plotted against the nominal analyte concentration. The units for calibration were picogram on column for normalization of liver tissue samples by milligram of tissue after conversion of the picogram on column to a nanogram per milliliter concentration. Calibration standards ranged 20.0 to 20,000 pg on column for PTV and 5.00 to 10,000 pg on column for RTV with linear $1/x^2$ weighted regression [25] using pooled blank rat liver homogenate supernatant as matrix.

Accuracy & precision

The interday and intraday accuracy and precision were measured using three control concentration sets of six prepared and frozen control samples each analyzed on three different days. The concentrations used were: 60.0, 4000 and 15,000 pg on column for PTV and 15.0, 2000 and 7500 pg on column for RTV designated as LQC, MQC and HQC, respectively and were prepared in blank rat liver homogenate. Accuracy and precision at the LLOQ was assessed at 20.0 and 5.00 pg on column for PTV and RTV and analyzed on three different days. The calculated mean concentration relative to the nominal concentration was used to express accuracy (%bias). The relative standard deviation (RSD) was calculated from the QC values and used to estimate the precision.

Matrix effects

Matrix effect (ME), process efficiency and recovery efficiency were tested for PTV and RTV and the internal standards as described previously [26,27]. Testing was performed using PTV and RTV at three different concentrations (LQC, MQC and HQC) in triplicate with five independent lots of rat liver homogenate. One set was prepared as a neat sample (no extraction), one set was prepared in rat liver homogenates after homogenization (postpreparative) and one set was prepared in rat liver homogenates spiked prior to homogenization (prepreparative). ME was calculated at each concentration level by dividing the mean peak area ratio of the

postpreparative by the neat samples. Recovery (RE) was calculated at each concentration level by dividing mean peak area ratios of prepreparative by the mean postpreparative samples. Processing efficiency (PE) was calculated by dividing mean peak area ratios of prepreparative sample at each concentration level by the neat samples. The variation of the slopes using the peak area ratios from the five different lots of liver homogenate were examined to investigate if matrix effects occur over the different concentrations. Matuszewski *et al.* suggest variation be limited to a relative standard deviation of 5% or less [26].

Sample stability

In this manuscript we studied stability of PTV and RTV spiked into blank liver homogenates at low and high QC levels in triplicate as well as in intact tissue. Bench-top stability was tested by analyzing extracted homogenate-based QC samples that were left out on the bench top at room temperature for 4 h before processing them for an analytical run. Postpreparative stability was determined for processed samples stored at 10°C within the autosampler, which were re-injected and analyzed against a new standard curve at 24-h postprocessing. Freeze–thaw stability was assessed for extracted homogenate-based QC samples that were subjected to three freeze–thaw cycles from –70°C (for more than 24 h) to room temperature (at least 2 h) prior to analysis. Long-term stability of stock solutions was evaluated by comparing freshly prepared stock solutions to stock solutions that were prepared and stored at –70°C for 360 days. The results of all tested samples were compared with the recovery from samples that were freshly prepared (100% control). Stability was defined as being within 10% of the respective nominal control value.

Stability of PTV and RTV was also assessed on intact liver tissue by collecting samples (sectioned tissue, 1, 3 or 5, FNA passes, as well as CNB) and allowing them to sit on the bench for 1 h before being snap frozen and comparing similarly collected samples that were snap frozen immediately. The rat in the stability study was dosed by oral gavage with 30 mg/kg PTV and 15 mg/kg RTV for 5 days. Two hours after the last dose, the rat was sacrificed and tissues were obtained as described in the application of the method section. Last, we evaluated the stability of liver homogenates that were stored at 4°C for 80 days. These samples were run on a freshly prepared standard curve and compared with the original analysis.

Application of the method

The method described was applied to samples collected from a rat's liver. The male Sprague–Dawley rat was

dosed by oral gavage with 30 mg/kg PTV and 15 mg/kg RTV (dissolved in water to form a suspension) for four consecutive days by oral gavage. On the fifth day, the rat was sacrificed 3 h after it received its fifth dose. Post thoracotomy, FNA (22G 3.5-inch spinal needle on a 10 cc syringe in a Belpo syringe holder), CNB (16G removing a 2 cm core) and remaining liver was collected and frozen at -70°C until analysis. FNA were performed by placing the 22G needle into the rat liver. Multiple oscillations are performed for 5–10 s under negative pressure. The needle is removed and the material in the needle bore is expunged. This entire procedure is called a single FNA pass and repeated twice more for the three-pass sample and four additional times for the five-pass sample. Rat liver samples prepared and analyzed included one CNB, three different FNA samples composing of a single-pass sample, a three-pass sample and a five-pass sample and last the remaining tissue that was assayed in triplicate from two differently sectioned liver portions. The protocol was approved by the University at Buffalo Institutional Animal Care and Use Committee (IACUC).

Results & discussion

In order to support studies exploring PK of drugs within liver, a simple and rapid UPLC–MS/MS bioanalytical method was developed and validated for simultaneously determining PTV and RTV in liver tissue. It was validated according to the FDA guidance for bioanalytical method validation [28]. The method was then applied to the measurement of PTV and RTV in rat liver samples to compare different methods of acquiring tissue.

Method development

The method described was an adaptation from an unpublished assay to measure PTV and RTV in plasma samples that was provided by the sponsor (AbbVie, Inc.). The biggest difference between measuring these drugs in plasma versus tissue is that the analytes need to be released from the tissue sample into a liquid in a fashion that does not compromise the integrity of the drug concentration. Experiments were performed by extracting the drugs from tissue using a bullet blender. Zirconium oxide beads were used to disrupt the tissue. After testing various buffers, solvents and mixtures of the two, acetonitrile was determined to be the best homogenization solution since it denatured the protein from the liver, and it stabilized the drugs from potential metabolism.

The ability to recover known amounts of PTV and RTV from varying tissue weights was tested to assess if the ratio of liver to acetonitrile in the homogenate matrix had any effect on analyte detection. To accomplish this

objective, various concentrations (LLOQ, MID and ULOQ) of PTV and RTV were spiked into 400 μl of acetonitrile (40 μl of a $10\times$ concentration into 360 μl acetonitrile) that was subsequently added to either 5, 10, 15, 20 or 30 mg of rat liver tissue. The rat liver used for these experiments was obtained from an animal that was not exposed to PTV or RTV (blank rat liver). Our results show that a consistent area ratio is observed without significant differences in the amount of drug recovered across the varying tissue amounts ranging from 5 to 30 mg (Figure 1, $p > 0.05$, one-way ANOVA). It is reassuring that the results are consistent across a variety of sample weights since the weight of samples obtained during the conduct of a human or animal study will also likely vary considerably by weight.

We next assessed the completeness of drug recovery from rat liver tissue that was dosed with PTV and RTV. Since it is not possible to inject the liver tissue with known amounts of the medications, we proceeded with the following steps to evaluate recovery, 320 μl (80%) of homogenate supernatant was removed from the centrifuged samples after they were homogenized with an expectation that 20% of the drug would remain. Then 320 μl of fresh acetonitrile was added back to the sample, we repeated the homogenization procedure and subsequently analyzed the re-extracted samples. When the samples were re-extracted, they simply underwent a second homogenization step to investigate if additional drug could be recovered. We found that the method is capable of extracting $98 \pm 1.9\%$ of PTV and $99 \pm 1.1\%$ of RTV in a single homogenization step proving that the developed tissue processing recovers virtually all PTV and RTV.

Chromatographic separation

Representative chromatograms of blank liver homogenate with and without internal standards, the LLOQ, and the rat that was dosed with PTV and RTV are shown in Figure 2. The blank liver homogenate shows the absence of analyte peaks and the blank liver homogenate with internal standard demonstrates acceptable purity with the lack of unlabeled analyte. The analytes were identified based on retention times and mass spectra of individual analytical standards. The retention times were approximately 1.03 and 2.09 min for RTV and PTV, respectively. The peaks of interest were well separated and without interference from other compounds. A total run time of 4.25 min allowed for equilibration of the column to the initial conditions.

Assay validation

Calibration & linearity

Calibration curves were obtained over concentration ranges of 20.0–20,000 pg on column for PTV and

Figure 1. Effect of tissue weight on the recovery of drug from rat liver tissue. PTV and RTV were spiked into the homogenization solution derived from three tissue samples of varying weights (either 5, 10, 15, 20 or 30 mg) and processed as described. Since no matrix-related trends were observed, we conclude that varying tissue mass (5–30 mg) does not affect the detection of the PTV or RTV in rat liver tissue samples. PTV: Paritaprevir; RTV: Ritonavir.

5.00–10,000 pg on column for RTV with a correlation coefficient (r^2) greater than 0.9931 for all curves. Calibration ranges were chosen based on the 2:1 ratio of dosing for the rat in the application section of this manuscript. Calibration curves were generated by weighted ($1/x^2$) linear regression analysis. During validation, we monitored accuracy and precision at the LLOQ concentration on three separate days with $n = 6$ each day. The results of this study are shown in Table 1 and confirm that the described method does not exhibit bias at lower concentrations with both accuracy and precision

less than 14.0%. Signal-to-noise between the lowest calibrator and blank liver homogenate was greater than 10:1 when compared with blank liver homogenate samples. Limit of detection (LOD) was 1.00 pg on column for both PTV and RTV. The intra- and inter-day accuracy and precision were $\leq 7.92\%$ for all levels of calibration standards.

Accuracy & precision

The intraday and interday accuracy (%bias) and precision (RSD) were determined at QC concentrations of

Figure 2. Representative chromatograms of a blank rat liver homogenate without internal standards added (double blank), a blank rat liver homogenate with internal standards, the LLOQ and a rat liver homogenate obtained from a sample collected at 3 h after the rat received 30 mg/kg PTV and 15 mg/kg RTV at steady state. Mass observed was 6756 pg on column for PTV and 3220 pg on column for RTV. PTV: Paritaprevir; RTV: Ritonavir.

Analyte	Level	Nominal concentration (pg on column)	Intraday (n = 6)		Interday (n = 18)	
			%Bias	RSD	%Bias	RSD
PTV	LLOQ	20.0	6.33	7.99	8.88	5.64
	LQC	60.0	9.17	4.11	10.1	4.72
	MQC	4000	-0.551	2.27	-1.27	2.55
	HQC	15000	-7.12	3.09	-6.68	3.95
RTV	LLOQ	5.00	5.11	6.50	14.0	9.22
	LQC	15.0	4.86	4.62	7.03	5.33
	MQC	2000	-2.44	0.591	-1.69	4.12
	HQC	7500	-6.03	1.36	-5.30	3.15

60.0, 4000 and 15,000 pg on column for PTV and 15.0, 2000 and 7500 pg on column for RTV. Assay accuracy (%bias) for both analytes ranged from -6.68 to 10.1%, and precision (RSD) ranged from 0.591 to 5.33% (Table 1).

Matrix effect

ME was evaluated by comparing analyte signal in the presence and absence of matrix (Table 2). ME of the peak area ratio ranged from 106 to 112% recovery of analyte from the matrix as compared with neat solutions. These results illustrate mild signal enhancement although less than 15% from neat solutions, indicating no significant matrix effect in the five sources of rat liver evaluated. RE ranged from 100 to 109% showing no significant loss of analyte. The method described does not have an extraction step so it is unlikely that significant analyte quantities would be lost as compared with a method utilizing either solid phase or liquid–liquid extraction. PE ranged from 110 to 119%. The goal of the matrix effect study was to compare spiking known concentrations before homogenization (prepreparative) versus spiking the liver homogenates (postpreparative) and to compare those to spiking solvent without liver homogenate to prove the integrity of the drugs throughout the sample processing steps and evaluate the phenomena of matrix effect within five lots of rat liver tissue. The ME, RE and PE were consistent over the three concentrations in the five different independent lots of rat liver evaluated. Slopes were generated by plotting the average ratio of peak to internal standard area versus the theoretical concentration and obtaining a best fit line from the three concentrations in each matrix. RSDs were calculated from the average of the slopes and found to be less than 3.59%.

There was no evidence of carryover for PTV, RTV or their internal standards as found by injecting mobile phase after the highest calibrator sample in a carry-over experiment. Random placement of mobile phase

blanks throughout a run also showed no evidence of carryover.

Sample stability

Sample stability recommendations are stated in the FDA guidelines but they only refer to plasma-based assays. Tissue samples can be assessed in two different ways: on intact tissue or on homogenized tissue supernatants. Intact tissues cannot be homogeneously spiked with analyte and therefore stability should be assessed on tissue homogenate spiked with analyte [29]. Bench-top, postpreparative and freeze–thaw stability were assessed for analytes at LQC and HQC levels. Samples were found to be stable for 4 h at room temperature (bench-top), and 24-h postanalysis at 10°C in the refrigerated autosampler. Additionally, three freeze–thaw cycles had no effect on the stability of analytes in the homogenized rat liver supernatant. The mean measured concentrations of analytes ranged from 94.0 to 103% of freshly

Table 2. Matrix effect of paritaprevir and ritonavir in rat liver homogenate (n = 5).

	Paritaprevir			Ritonavir		
	PTV	PTV-IS	RATIO	RTV	RTV-IS	RATIO
ME (post/neat)						
LQC	87.2	79.6	110	97.9	88.6	112
MQC	88.2	83.1	106	98.0	92.9	106
HQC	93.3	84.5	110	102	95.2	108
RE (pre/post)						
LQC	102	95.3	107	108	102	106
MQC	103	94.2	109	109	101	107
HQC	100	100	100	106	102	104
PE (pre/neat)						
LQC	89.1	75.9	118	106	90.2	119
MQC	90.6	78.3	116	106	93.8	113
HQC	92.8	84.2	110	109	96.8	112

analyzed samples, indicating adequate stability under all conditions tested. PTV stock solution was tested as stable at -70°C for 360 days in acetonitrile within an amber glass vial. RTV stock solution was tested previously in our laboratory as stable in methanol in an amber glass vial at -70°C for 6 years. The drug stability of PTV and RTV in liver tissue showed no significant difference between immediately snap frozen (fresh) versus the liver tissue being set on a bench for 1 h before snap freezing (stability) as shown in Figure 3. The CNB

sample for PTV had a 50% decrease ($n = 1$) between immediate snap freeze and being set on a bench for 1 h before snap freezing samples but there was only one sample to compare. The stability in homogenate supernatant stored at 4°C for 80 days was 102 and 93.1% for PTV and RTV, respectively.

Application of the method

The method was applied to the measurement of concentrations of PTV and RTV in liver tissue samples

Figure 3. Stability of paritaprevir and ritonavir in sectioned rat liver tissue ($n = 6$), FNA ($n = 3$, mean of one-, three- and five-pass FNA samples) and CNB samples ($n = 1$). Fresh samples were snap frozen immediately after collection while stability samples were left on the bench top for 1 h after collection before snap freezing. CNB: Core needle biopsy; FNA: Fine needle aspirate.

obtained from a rat using procedures identical to those used in humans: sectioned tissue (two different sections in triplicate), CNB ($n = 1$) and FNA ($n = 1$ per pass pool). Figure 4 shows the differences observed in recovered drug concentration among the sampling techniques. Less drug was recovered from CNB ($n = 1$) and FNA pools (pools = 1, 3 and 5 passes) as compared with the tissue section samples ($n = 3$ sections per sample). The mean or individual drug concentrations (nanogram drug per milligram of tissue weight) from each type of liver sample are shown in Table 3. A possible explanation for our results is that smaller tissue samples are prone to greater variability in weight, which we used to normalize the drug concentration as an example, FNA samples have noticeably greater amount of blood in comparison with the other sample types [30]. The blood contamination in the sample, although small, impacts the weight of the sample in addition to its drug content. Aside from the weight of the blood, the concentration ratio in the blood versus the tissue may impact the results when the partitioning is unequal. For example, the presence of blood may lower the drug concentration in the tissue if the drug was mostly in tissue versus blood. It appears that the tissue sections yield the most accurate and precise drug concentration because they are more consistent from sample to sample. However, they are also the most invasive and are not feasible for serial sampling in humans.

Conclusion & future perspective

We have developed and validated a novel UPLC–MS/MS method for the simultaneous determination of PTV and RTV in rat liver tissue. The assay incorporates simple sample processing, a short run-time and results in excellent accuracy, precision and recovery. These results validate the assay as well suited for high-throughput

Figure 4. Paritaprevir and ritonavir recovered from rat liver tissue obtained by sectioning ($n = 6$), fine needle aspiration ($n = 3$) and core needle biopsy ($n = 1$). Sectioned tissue yielded 39.6 ± 3.31 ng/mg PTV and 19.4 ± 0.937 ng/mg RTV. FNA yielded 21.3 ± 6.53 ng/mg PTV and 10.8 ± 2.29 ng/mg RTV. CNB yielded 25.1 ng/mg PTV and 14.0 ng/mg RTV.

CNB: Core needle biopsy; FNA: Fine needle aspirate; PTV: Paritaprevir; RTV: Ritonavir.

routine clinical or research purposes. The method is currently being used to support investigation of the PK of these compounds in liver tissue. Understanding the liver PK of drugs used to treat hepatitis C is paramount to their effectiveness since the best outcomes are accomplished when the drug is present in concentrations that lead to undetectable viral loads. This study in a rat was set up as a pilot investigation to evaluate the possibility of measuring the drugs in liver biopsies. The goal was to determine if FNA biopsy could yield enough tissue to give a drug concentration and to determine if multiple passes by FNA were required to get extra tissue if one pass was insufficient. Future plans are to study the 3D regimen of paritaprevir, ombitasvir and dasabuvir in human samples and therefore we seek to discover the least invasive mechanism to obtain a liver sample for drug PK analysis.

Table 3. Concentration of paritaprevir and ritonavir observed in liver biopsies.

Sample	Mass on column (pg)		Homogenate conc. (ng/ml) [†]		Weight (mg)	Tissue conc. (ng/mg) [‡]	
	PTV	RTV	PTV	RTV		PTV	RTV
CNB	2182	1215	546	304	8.68	25.1	14.0
FNA (1 pass)	1434	820	359	205	8.99	16.0	9.13
FNA (3 pass)	6052	2838	1513	710	21.2	28.6	13.4
FNA (5 pass)	3960	2018	990	505	20.5	19.3	9.83
Tissue 1 [§]	6959 ± 1067	3387 ± 510	1740 ± 267	847 ± 128	17.0 ± 1.99	40.9 ± 2.41	19.9 ± 0.862
Tissue 2 [§]	4809 ± 137	2376 ± 102	1202 ± 34.3	594 ± 25.5	12.6 ± 1.04	38.3 ± 4.10	18.9 ± 0.833

[†]To convert from picogram on column, to nanogram per milliliter, divided by 4 to account for the volumes used in sample processing.

[‡]To convert from nanogram per milliliter to nanogram per milligram, multiplied by 0.4 and divided by milligram tissue weight to account for the amount of tissue and volume in the homogenized sample.

[§]Values shown as mean ± standard deviation, $n = 3$.

CNB: Core needle biopsy; FNA: Fine needle aspirate; PTV: Paritaprevir; RTV: Ritonavir.

Future perspectives will include the development of sampling techniques to be further developed for example: FNA collections, the liver tissue obtained should be separated from any blood that was acquired during the aspiration by low-speed centrifugation. Stability in tissue, to evaluate any potential loss of drug concentration over time.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the NIH.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Measurement of hepatitis C virus direct-acting antivirals in liver tissue is of great importance because the liver is the site of action for paritaprevir (PTV).

Experimental

- Liver biopsy samples were obtained from a rat dosed with 30 mg/kg PTV and 15 mg/kg ritonavir (RTV).
- Liver tissue was homogenized in the presence of acetonitrile to denature protein and stabilize further drug metabolism.
- Liver homogenate supernatants were analyzed for drug concentration by UPLC–MS/MS.
- A simple UPLC–MS/MS method was developed and validated to measure PTV and RTV in rat liver tissue.

Results

- Validation of the method according to the US FDA guidelines proved robustness by demonstrating great accuracy and precision.
- Matrix effect was minimized by using deuterated internal standards for PTV and RTV (d8-PTV and d6-RTV).

Conclusion

- The method can be used to study PK in liver tissue by sectioning, fine needle aspirate and core needle biopsies.

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Papers of special note have been highlighted as:

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