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Development and Full Validation of a Bioanalytical Method for Quantifying Letemovir in Human Plasma Using Ultra-Performance Liquid Chromatography Coupled with Mass Spectrometry

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With the aim of studying the pharmacokinetics of letemovir, which is a newly developed antiviral agent for human cytomegalovirus, a rapid and simple ultra-performance liquid chromatography coupled with mass spectrometry (UPLC/MS) method was developed and validated for the quantification of letemovir in human plasma. Separation was performed in reverse phase mode using an ACQUITY UPLC BEH C18 column (130 Å, 1.7 μm, 2.1 × 50 mm) at a flow rate of 0.3 mL/min, 10 mM ammonium acetate–0.1% formic acid solution as mobile phase A, and acetonitrile as mobile phase B with a gradient elution. The method was validated over a linear range of 10–1000 ng/mL with a coefficient of determination (R^2) > 0.99 using weighted linear regression analysis. The intra- and inter-assay accuracy (nominal%) and precision (relative standard deviation%) were within ±15 and ≤15%, respectively. The specificity, recovery, matrix effect, stability, and dilution integrity of this method were also within acceptable limits. This method could be useful in studying the pharmacokinetics and pharmacodynamics, as well as performing the therapeutic drug monitoring of letemovir.

Key words letemovir; bioanalytical method development; validation; ultra-performance liquid chromatography coupled with mass spectrometry; human plasma

Introduction

Letemovir is a newly developed antiviral agent indicated in the prophylaxis of human cytomegalovirus (CMV) infections in adult recipients of allogeneic hematopoietic stem cell transplants (HSCT).^{1–3} Unlike other antiviral agents such as ganciclovir, foscarnet, and cidofovir, which target the viral DNA polymerase,⁴ letemovir inhibits viral DNA cleavage by targeting the CMV terminase complex, pUL56.⁵ It is commercially available in both intravenous and oral formulations with a recommended therapeutic dose of 480 mg once daily.⁶

Recently, several studies have reported various cases of CMV resistance during letemovir prophylaxis in HSCT recipients.^{7–9} Furthermore, letemovir was found to increase tacrolimus exposure by inhibiting CYP3A4^{10,11} and decrease voriconazole exposure in healthy recipients secondary to CYP2C9/19 induction.¹² It has also been found that co-administration with cyclosporine significantly decreases letemovir clearance, leading to an increase in letemovir exposure caused by the inhibitory effect of cyclosporine on organic anion transporting polypeptide (OATP)1B1/3 transporters; OATP1B1/3 is a hepatic metabolizer of letemovir.¹¹

In light of these findings, further studies for assessing the pharmacokinetics (PK) and pharmacodynamics (PD) are required. However, although multiple clinical studies^{3,10,11,13} mention the use of LC/MS methods for quantifying letemovir in human plasma, there is no information regarding the full method, including its validation in the available literature. More recently, a new method for the quantification of letemovir in human serum using HPLC combined with diode array detector has been published.¹⁴ However, to date, no LC/MS

method has been described in human plasma. Thus, the main purpose of this work was to develop and validate an ultra-performance liquid chromatography (UPLC)/MS method for quantifying letemovir in human plasma, which is suitable for clinical studies as well as for therapeutic drug monitoring.

Experimental

Chemicals and Reagents The reference standard letemovir (≥98% purity) was purchased from Cayman Chemical (Ann Arbor, Michigan, U.S.A.). The chemical structure of letemovir is presented in Fig. 1.⁵ Roscovitine, which was used as the internal standard (IS), was purchased from Wako Pure Chemical Corporation (Osaka, Japan). Methanol (MeOH), acetonitrile (ACN), dimethylsulfoxide (DMSO, 99% purity), and formic acid (98% purity) were supplied by Wako Pure Chemical Corporation. Methanol and acetonitrile were both

Fig. 1. Chemical Structure of Letemovir (C₂₉H₂₈F₄N₄O₄)⁵

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LC/MS grade. Ammonium formate (1 mol/L) and ammonium acetate (10 mol/L) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Milli-Q water (of 18.2 m Ω) was obtained from an in-house purification system, Elga LabWater PURELAB[®] flex (Elga LabWater, High Wycombe, U.K.). Human blank plasma K2 dipotassium ethylenediaminetetraacetic acid (EDTA) was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan). Letermovir reference standard and human blank plasma were stored at -20°C prior to use.

Equipment The LC system consisted of an ACQUITY UPLC[®] system, equipped with an autosampler, a binary solvent manager, and an online degasser. Analyte separation was performed in reverse phase mode with an ACQUITY UPLC[®] BEH C18 column (130 Å, 1.7 μm , 2.1 $\times$ 50 mm) preceded by Waters ACQUITY BEH C18 Van-Guard pre-column and detected using Waters ACQUITY[®] QDa detector. The chromatography system, column, and mass spectrometer were purchased from the Waters Corporation (Milford, MA, U.S.A.). Data manipulation was performed using the Empower 3 software (Waters Corporation).

Method Development During method development, a large number of chromatographic and mass spectrophotometric parameters were screened and optimized by using a screening protocol, in order to develop a fast and robust bioanalytical method for quantifying letermovir in human plasma.

MS Conditions

Detection of letermovir and IS, based on the peaks' mass to charge (m/z) ratio, was carried out using Waters ACQUITY[®] QDa detector through electrospray ionization (ESI) operating in a positive ion mode. The cone and capillary voltages were 15 V and 0.8 kV, respectively. The probe temperature was set to 600°C , and the sampling rate was set to 10 points/s.

Chromatographic Conditions

Chromatographic separation was performed in reverse phase mode with an ACQUITY UPLC BEH C18 column maintained at 50°C . The mobile phase was delivered at a flow rate of 0.3 mL/min through gradient elution and consisted of 10 mM ammonium acetate in pure water with 0.1% formic acid added for pH adjustment (aqueous mobile phase A) and acetonitrile (organic mobile phase B). The total analytical run time for each injection was 6.1 min, including 2 min of re-equilibration.

The initial gradient conditions started with 10% mobile phase B which was maintained for 0.5 min. Mobile phase B was gradually increased to 40% over 1 min and further increased to 90% for 1.4 min. These conditions were held for 1.1 min, returned to initial conditions over 0.1 min, and maintained for 2 min. Optimization of chromatographic conditions involved the consecutive screening of the following parameters: buffer, pH of the mobile phase, organic solvent, gradient elution, flow rate, column temperature, and injection volume. The initial method conditions for the first screening were selected according to letermovir physicochemical properties (pK_a values, molecular weight, solubility, and stability). The conditions offering the best retention and separation were then selected.

Preparation of the Stock Solution, Calibration Standards, and Quality Control (QC) Working Solutions

Stock solution was prepared at 1 mg/mL by dissolving 5 mg of letermovir standard in DMSO. The stock solution was further diluted in an appropriate volume of methanol–water (1 : 1, v/v) to prepare a second stock solution at 100 $\mu\text{g/mL}$. From the

second stock solution, two individual working solutions (calibration standard working solution and QC working solutions) were freshly prepared in methanol–water (1 : 1, v/v) at 10 and 7.5 $\mu\text{g/mL}$, respectively. Serial dilution with methanol–water (1 : 1, v/v) was then performed in order to obtain six calibration standard working solutions and three QC working solutions. The IS working solution was prepared at 5 $\mu\text{g/mL}$ by diluting the corresponding stock solution in methanol–water (1 : 1, v/v).

Calibration Standards, and QC Samples

Calibration standard working solutions were diluted 10-fold with human blank plasma (1 : 9, v/v) to obtain six calibration standards at 10, 100, 200, 400, 800, and 1000 ng/mL. QC working solutions were also diluted 10-fold with human blank plasma to obtain QCs at three different concentration levels: i.e., low-, mid-, and high-: levels of 30, 500, and 750 ng/mL, respectively.

Samples Pre-treatment

Ten microliters of the IS working solution were added to 1.5 mL centrifuge polyethylene tubes containing 100 μL of the corresponding calibration standard or QC diluted in human blank plasma. Protein precipitation was induced by adding 400 μL of methanol to all the samples. The tubes were vortexed for 1 min and centrifuged at $13000 \times g$ for 5 min. The supernatant was diluted with an appropriate volume of mobile phase (1 : 1, v/v), and 2 μL was injected into the LC/MS system.

Method Validation Full validation of the method in human plasma was performed in compliance with the U.S. Food and Drug Administration (FDA) guidelines on bioanalytical method development and validation.¹⁵⁾ The validation included the following parameters: linearity and calibration curve, carry over, selectivity, intra-, and inter-assay accuracy and precision, recovery and matrix effect, dilution linearity, and stability.¹⁶⁾

Linearity and Calibration Curve

Calibration standards were prepared at six concentrations over a range of 10–1000 ng/mL. Six individually prepared replicates at each concentration, including a blank and a zero calibrator standard (blank plasma spiked with IS), were analyzed within six different runs. In order to confirm the concentration-response relationship, calibration curves were plotted by fitting the peak response (y -axis), defined as the peak-area ratio of letermovir to IS, against the nominal concentration of the calibration standard (x -axis).

Concentrations were calculated using unweighted and weighted linear least-squares regression analysis with the weighting factors: 1, $1/x$, and $1/x^2$, where x is the concentration of letermovir. The choice of the weighting factor was based on the relationship between the standard deviation and variance of the LC/MS response and the analyte concentration.¹⁷⁾

The coefficient of determination (R^2) should be ≥ 0.99 . Accuracy (nominal%) and precision (relative standard deviation (RSD) %) at each concentration should be $\pm 15\%$ and $\leq 15\%$, respectively, except at the lower limit of quantification (LLOQ), where the calculated concentration should be $\pm 20\%$ of the nominal concentration and $\text{RSD} \leq 20\%$.

Carryover

Carryover was evaluated by analyzing a human blank plasma following the injection of an upper limit of quantification (ULOQ), the highest concentration calibration standard, in five different runs. Carryover should not exceed 20% of the

LLOQ.

Selectivity

Selectivity was defined as the absence of substances interfering with the analyte and IS at the same retention time and was evaluated by analyzing six different human plasma samples prepared at LLOQ (10 ng/mL) and IS at 500 ng/mL. The human plasma samples should be free of interferences with the elution of the analyte and IS. If any peak is observed at the retention time of the analyte and/or IS, the response should be $\leq 20\%$ of the LLOQ response and $\leq 5\%$ of the response of the IS.

Accuracy and Precision

Accuracy and precision were evaluated at LLOQ level, and with QCs prepared at three different concentrations. Repeatability or intra-assay accuracy, defined as nominal%, and precision (RSD%), were determined by analyzing five individually prepared replicates at each concentration within the same run and five injections of one replicate within another run to evaluate injection repeatability.

Inter-assay accuracy and precision were obtained by analyzing five individually prepared replicates at each concentration within five different days. Accuracy at each concentration level should be within $\pm 15\%$, and precision should be $\leq 15\%$, except at the LLOQ where accuracy and precision should be ± 20 and $\leq 20\%$, respectively.

Matrix Effect and % Recovery

QCs at low, mid, and high levels were prepared in six different human plasma samples. The matrix factor was evaluated by comparing the peak area of the QC prepared in human plasma with the peak area of the QC prepared in methanol–water (1 : 1, v/v).

Recovery was evaluated by comparing the peak area of the extracted QC prepared at three levels of concentration with the peak area of the extracted human plasma sample spiked with letermovir and IS at the same concentration. Precision expressed by the RSD% for both the matrix effect and recovery should not exceed 15%.

Dilution Linearity

Plasma dilution in human blank plasma was assessed. Five individually prepared replicates of QCs at 10 times (10 $\mu\text{g/mL}$) the ULOQ were analyzed. The samples were diluted 10 times with human blank plasma to obtain samples of 1000 ng/mL. The mean accuracy at each level of concentration should be within $\pm 15\%$, and the precision expressed in RSD% should be $\leq 15\%$.

Stability

The stability of the stock solution was assessed by analyzing three individually prepared replicates in methanol–water (1 : 1, v/v) at 1 $\mu\text{g/mL}$, after storage at room temperature (r.t., 24°C) for 6 h, and after storage at -20°C for 6 months and 12 months. The stability of letermovir in human blank plasma was determined using five individually prepared replicates

of QCs at three concentration levels. The following stability conditions were assessed: short-term stability (24 h at r.t. and at -20°C), post-preparation (48 h at -20°C), auto-sampler (24 h at 10°C), freeze thaw (three cycles, $-20^\circ\text{C}/\text{r.t.}$, and 24 h between cycles), and long-term stability (10 d at -20°C , and 30 d at -20 and -80°C). Letermovir was considered stable under the different storage conditions if the accuracy (nominal%) and precision (RSD%) were within ± 15 and $\leq 15\%$, respectively.

Results and Discussion

Method Development

Mass Spectrometry

The ionization conditions used in the full mass scan permitted the detection of prominent peaks for letermovir and IS with m/z of 573.30 and 355.31, respectively. Single chromatograms of letermovir were obtained by setting the QDa mass spectrometer to single m/z values previously obtained through a selected ion recording (SIR). The results of the full mass scan and SIR showed that no further optimization was required. Thus, initial ESI-MS was selected for optimizing the chromatographic conditions.

LC

The chromatographic method was optimized by changing various parameters such as pH of the buffer and organic solvent. Best separation, elution, and tailing were obtained with 10 mM ammonium acetate in pure water and 0.1% formic acid to obtain a pH of 5.2 (mobile phase A) and acetonitrile (mobile phase B). Furthermore, to achieve a capacity factor ≥ 2 , the column temperature was maintained at 50°C and acetonitrile was gradually increased from 10 to 90%, over 2.9 min.

Method Validation

Linearity and Calibration Curve

The equations of linear regression and coefficients of determination R^2 are described in Table 1. The concentration–response relationship was best described with a $1/x^2$ weighting factor. R^2 of six replicates was > 0.99 , and the accuracy of all six calibration points was within $\pm 15\%$ of the nominal concentration. Thus, the calibration curve was validated over a range of 10–1000 ng/mL.

The choice of the upper limit of 1000 ng/mL was based on the results of previous clinical trials.^{3,11)} The predicted median minimal concentration of letermovir in Asian patients after oral administration of 480 mg, and after oral administration of 240 mg with cyclosporine exposure were 506.4 ng/mL with a 90% prediction interval of 118.4–1628, and 1200 ng/mL with a 90% prediction interval of 346.9–3154, respectively.³⁾ Furthermore, according to a study conducted by Isberner *et al.*, the median trough concentration of letermovir after oral administration of 240 or 480 mg in patients including children was 2603 ng/mL (range 175–12281 ng/mL), and two samples were less than 100 ng/mL.¹⁴⁾ These results suggest that letermovir

Table 1. Calibration Curve and Regression Parameters Results Obtained with Unweighted and Weighted Linear Regression Analysis^{a)}

Weighting factor	Mean equation for regression line	Mean coefficient of determination (R^2)	Mean RE% ^{b)}
1	$y = 0.000873x + 0.002119$	0.9995	–4.8
$1/x$	$y = 0.000879x - 0.000336$	0.9997	3.3×10^{-3}
$1/x^2$	$y = 0.000882x - 0.000484$	0.9995	5.0×10^{-2}

^{a)} Regression parameters obtained with calibration curves of six standards in six analytical runs. ^{b)} Mean relative error (RE)%: ((obtained concentration – nominal concentration)/nominal concentration) $\times 100$

concentrations exhibit a large variability in clinical setting. In this study, a lower ULOQ than that used in a previous study (5000 ng/mL)¹⁴⁾ was chosen to improve the accuracy of our calibration curve, while a dilution integrity of 10-fold was assessed in order to account for concentrations above 1000 ng/mL.

Carryover

No response was detected in the five replicates of human blank plasma samples processed after a ULOQ sample at the retention time of letermovir and IS. Thus, no carry-over effect was observed.

Fig. 2. Chromatograms of Letermovir and IS in Human Plasma; (A) Blank Plasma $m/z = 573.30$; (B) Blank Plasma Spiked with Letermovir at LLOQ (10 ng/mL); (C) Blank Plasma $m/z = 355.31$; (D) Blank Plasma Spiked with IS (500 ng/mL)

Table 2. Intra- and Inter-Assay Accuracy and Precision of Letermovir QC Samples

QC level	Nominal concentration (ng/mL)	Intra-assay ($n = 5$) ^b		Intra-assay ($n = 5$) ^c		Inter-assay ($n = 5$)	
		Accuracy (%) ^a	Precision (RSD%)	Accuracy (%)	Precision (RSD%)	Accuracy (%)	Precision (RSD%)
LLOQ	10	103	2.6	92	2.0	106	4.5
Low QC	30	99	3.3	110	1.2	99	3.0
Mid QC	500	101	1.9	108	0.4	100	3.6
High QC	750	102	1.7	106	0.4	100	2.3

LLOQ, lower limit of quantification; QC, quality control. *a*) Accuracy: (obtained concentration/nominal concentration) $\times$ 100, *b*) Five individually prepared replicates, *c*) Five injections of one replicate.

Table 3. Matrix Effect ($n = 6$) and Recoveries of Letermovir and IS from Human Plasma ($n = 6$)

	QC level	Nominal concentration (ng/mL)	Matrix effect		Recovery	
			Absolute matrix factor ^a	Precision (RSD%)	Recovery (%) ^b	Precision (RSD%)
Letermovir	Low QC	30	1.5	8.5	93	8.0
	Mid QC	500	1.3	4.0	97	4.8
	High QC	750	1.3	3.3	94	4.9
Roscovitine (IS)		500	1.0	5.4	101	5.7

QC, quality control. *a*) Absolute matrix factor: (peak area of analyte in human plasma/peak area of analyte in neat solvent). *b*) Recovery%: (peak area of analyte in pre-spiked plasma/peak area of analyte in post-spiked plasma) $\times$ 100

Selectivity

The method showed good selectivity for letermovir and IS. The different chromatograms are presented in Fig. 2. Retention times of letermovir and IS were 2.78 and 2.69 min, respectively. No endogenous peak was observed in the human plasma samples at the retention times of letermovir and IS.

Sensitivity

Sensitivity was evaluated with five LLOQ samples (10 ng/mL) as per the intra-assay accuracy and precision (Table 2). The results of the five replicates met the acceptance criteria of accuracy (nominal% $\pm 15\%$) and precision (RSD% $\leq 15\%$). The LLOQ determined in this assay (10 ng/mL) was lower than the LLOQ established in a previous study by Isberner *et al.* (100 ng/mL).¹⁴⁾

Accuracy, Precision, and Dilution Linearity

Table 2 summarizes the results of the intra-assay and inter-assay accuracy and precision. The accuracy of all the QCs at different levels was within $\pm 15\%$, and precision was $\leq 15\%$. Both the methods used in our study and in a previous study¹⁴⁾ conformed with the FDA guidelines for bioanalytical method validation, in terms of accuracy and precision.¹⁵⁾ Dilution linearity with human blank plasma was investigated at a ratio of 1:9. The mean accuracy and precision values obtained were 103 and 0.5%, respectively. All the results met the described acceptance criteria, suggesting that samples with a concentration >1000 ng/mL can be quantified with good accuracy and precision.

Matrix Effect and Recovery

The results of the matrix effect and extraction recoveries of letermovir and IS from human plasma samples are presented in Table 3. The mean extraction recoveries and RSD% at three levels of QCs were ± 15 and $\leq 15\%$, respectively, demonstrating a good recovery in different human plasma samples. The RSD% value of the matrix effect expressing the inter-subject variability was $\leq 15\%$. Furthermore, the RSD% of mean recovery and matrix effect of IS were also $\leq 15\%$. These results

suggest that simple protein precipitation with methanol, compared to liquid–liquid extraction used by Isberner *et al.*,¹⁴⁾ is efficient for pre-treatment of the samples and contributes to optimized sample preparation time while achieving a good recovery during extraction.

Stability

The results of the stability studies are summarized in Tables 4 and 5. Letermovir stock solutions were stable for at least 1 year when prepared in a neat solvent. Furthermore, nominal% of QCs at three concentration levels after 30 d at -20 and -80°C were within $\pm 15\%$. Thus, letermovir is stable for at least 30 d in human plasma under the described storage conditions.

Conclusion

A rapid, simple, and sensitive bioanalytical method for the quantification of letermovir in human plasma was developed and validated in accordance with FDA guidelines.¹⁵⁾ This method constitutes the first described LC/MS method for the quantification of letermovir in human plasma. The advantage of this method lies in its short analysis time of 6.1 min compared to 20 min in a previous HPLC assay with a retention time of 5.79 ± 0.15 min.¹⁴⁾ Moreover, it has a simple method of sample preparation and high sensitivity following the use of a mass spectrometer detector through a single ion recording. The assay was fully validated with good selectivity and linearity over a large range of 10–1000 ng/mL. Letermovir was stable in neat solvent and plasma for at least 1 year and 30 d, respectively. A limitation of this study is the absence of clinical application on plasma samples of patients receiving letermovir treatment. Thus, an assessment of the assay using patients' samples is required.

In conclusion, the method was successfully used to quantify letermovir in human plasma. This work could be useful to perform not only clinical PK/PD studies but also to investigate drug–drug interactions and letermovir side effects, which

Table 4. Stability Results of Letemovir in Human Plasma at Different Conditions ($n = 5$)

Stability condition	Nominal concentration (ng/mL)	Nominal ^{a)} (%)	Precision (RSD%)
Auto sampler (10 °C, 24h)	30	106	2.3
	500	104	2.2
	750	103	1.4
Freeze-thaw Three cycles (−20 °C/r.t., 24h)	30	101	4.4
	500	103	5.4
	750	105	3.2
Post-preparation (−20 °C, 48h)	30	102	3.7
	500	98	1.7
	750	94	2.0
Room temperature 24 °C, 24h	30	99	3.1
	500	99	3.6
	750	96	3.0
−20 °C, 24h	30	110	6.0
	500	102	4.3
	750	104	2.7
−20 °C, 10 d	30	91	2.8
	500	96	1.7
	750	98	1.4
−20 °C, 30 d	30	112	5.4
	500	111	2.6
	750	110	2.8
−80 °C, 30 d	30	110	6.1
	500	110	2.3
	750	108	5.1

a) Nominal% = (response at the stability condition/response of freshly prepared sample) × 100

Table 5. Stability Results of Letemovir Stock Solution at Different Conditions ($n = 3$)

Stability condition	Nominal concentration (ng/mL)	Nominal (%) ^{a)}	Precision (RSD%)
Room temperature, 6h	1000	97	4.1
−20 °C, 6 months	1000	90	2.6
−20 °C, 12 months	1000	87	0.9

a) Nominal% = (peak area of letemovir at stability condition/peak area of freshly prepared stock solution) × 100

will be instructive in the creation of a dosage regimen and optimization of letemovir safety and efficiency.

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Conflict of Interest The authors declare no conflict of interest.

References

- 1) Chemaly R. F., Ullmann A. J., Stoelben S., Richard M. P., Bornhauser M., Groth C., Einsele H., Silverman M., Mullane K. M., Brown J., Nowak H., Kolling K., Stobernack H. P., Lischka P., Zimmermann H., Rubsamen-Schaeff H., Champlin R. E., Ehninger G., Team A. I. C. S., *N. Engl. J. Med.*, **370**, 1781–1789 (2014).
- 2) Goldner T., Hewlett G., Ettischer N., Ruebsamen-Schaeff H., Zimmermann H., Lischka P., *J. Virol.*, **85**, 10884–10893 (2011).
- 3) Marty F. M., Ljungman P., Chemaly R. F., *et al.*, *N. Engl. J. Med.*, **377**, 2433–2444 (2017).
- 4) Schreiber A., Harter G., Schubert A., Bunjes D., Mertens T., Michel D., *Expert Opin. Pharmacother.*, **10**, 191–209 (2009).
- 5) Lischka P., Hewlett G., Wunberg T., Baumeister J., Paulsen D., Goldner T., Ruebsamen-Schaeff H., Zimmermann H., *Antimicrob. Agents Chemother.*, **54**, 1290–1297 (2010).
- 6) Prevymis™ [Package insert]. Merck & Co., Inc., Whitehouse Station, NJ.: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/209939s005,209940s0031bl.pdf, cited 14 November, 2019.
- 7) Frietsch J. J., Michel D., Stamminger T., Hunstig F., Birndt S., Schnetzke U., Scholl S., Hochhaus A., Hilgendorf I., *Mediterr. J. Hematol. Infect. Dis.*, **11**, e2019001 (2019).
- 8) Jung S., Michel M., Stamminger T., Michel D., *BMC Infect. Dis.*, **19**, 388 (2019).
- 9) Komatsu T. E., Hodowanec A. C., Colberg-Poley A. M., Pikis A., Singer M. E., O'Rear J. J., Donaldson E. F., *Antiviral Res.*, **169**, 104549 (2019).
- 10) Kropit D., von Richter O., Stobernack H. P., Rubsamen-Schaeff H., Zimmermann H., *Clin. Pharmacol. Drug. Dev.*, **7**, 9–21 (2018).
- 11) McCrea J. B., Macha S., Adedoyin A., Marshall W., Menzel K., Cho C. R., Liu F., Zhao T., Levine V., Kraft W. K., Yoon E., Panebianco D., Stoch S. A., Iwamoto M., *J. Clin. Pharmacol.*, **59**, 1331–1339 (2019).
- 12) Marshall W. L., McCrea J. B., Macha S., Menzel K., Liu F., van Schanke A., de Haes J. I. U., Hussaini A., Jordan H. R., Drexel M., Kantesaria B. S., Tsai C., Cho C. R., Hulskotte E. G. J., Buttertont J. R., Iwamoto M., *J. Clin. Pharmacol.*, **58**, 897–904 (2018).
- 13) Kropit D., Scheuenpflug J., Erb-Zohar K., Halabi A., Stobernack H. P., Hulskotte E. G. J., van Schanke A., Zimmermann H., Rubsamen-Schaeff H., *Br. J. Clin. Pharmacol.*, **83**, 1944–1953 (2017).
- 14) Isberner N., Aghai F., Schirmer D., Kraus S., Grigoleit G. U., Klinker H., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, **1159**, 122399 (2020).
- 15) U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, “Guidance for industry: Bioanalytical method validation.” May 2018: <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf>, cited 10 October, 2019.
- 16) Sakurai N., Nakamura Y., Kawaguchi H., Abe J., Yamada K., Nagayama K., Kakeya H., *Chem. Pharm. Bull.*, **67**, 439–444 (2019).
- 17) Gu H., Liu G., Wang J., Aubry A. F., Arnold M. E., *Anal. Chem.*, **86**, 8959–8966 (2014).