Journal of Chromatography B, 878 (2010) 1513–1518

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Quantification of CLR1401, a novel alkylphosphocholine anticancer agent, in rat plasma by hydrophilic interaction liquid chromatography–tandem mass spectrometric detection

Hongliang Jiang[a,*], Michelle J. Cannon[a], Maria Banach[b], Anatoly N. Pinchuk[b], Giangthy N. Ton[b], Christie Scheuerell[a], Marc A. Longino[b], Jamey P. Weichert[b], Richard Tollefson[a], William R. Clarke[b], Qin C. Ji[a], Xiangyu Jiang[a]

[a] Department of Bioanalytical Chemistry, Covance Laboratories Inc., 3301 Kinsman Boulevard, Madison, WI 53704, USA
[b] Cellectar Inc., 3301 Agriculture Drive, Madison, WI 53716, USA

ARTICLE INFO

Article history:
Received 22 December 2009
Accepted 3 April 2010
Available online 10 April 2010

Keywords:
CLR1401
LC–MS/MS
Liquid–liquid extraction
Hydrophilic interaction chromatography
Alkyl phosphocholine

ABSTRACT

A rapid and specific LC–MS/MS based bioanalytical method was developed and validated for the determination of 18-(*p*-iodophenyl)octadecyl phosphocholine (CLR1401), a novel phosphocholine drug candidate, in rat plasma. The optimal chromatographic behavior of CLR1401 was achieved on a Kromasil silica column (50 mm × 3 mm, 5 μm) under hydrophilic interaction chromatography. The total LC analysis time per injection was 2.8 min with a flow rate of 1.5 mL/min under gradient elution. Liquid–liquid extraction in a 96-well format using ethyl acetate was developed and applied for method validation and sample analysis. The method validation was conducted over the curve range of 2.00–1000 ng/mL using 0.0500 mL of plasma sample. The intra- and inter-day precision and accuracy of the quality control samples at low, medium, and high concentration levels showed ≤ 5.9% relative standard deviation (RSD) and −10.8 to −1.4% relative error (RE). The method was successfully applied to determine the toxicokinetics of CLR1401 in rats from three dose groups of 0.4, 4.0, and 10.0 mg/kg/day via intravenous administration.

1. Introduction

CLR1401 (Fig. 1), 18-(*p*-iodophenyl)octadecyl phosphocholine, is an investigational drug candidate under development for the treatment of malignant tumors. CLR1401 is structurally classified as an alkylphosphocholine. This new class of drugs has been reported with significant antineoplastic and antiprotozoal activity [1–5]. Derived from long-chain alcohols, alkylphosphocholines lack the glycerol backbone of alkyl-lysophospholipid analogs and thus are not subject to metabolism by many of the phospholipid metabolizing cellular enzymes. In contrast, alkylphosphocholines alter metabolism and accumulate in tumors and other tissues [6,7]. In addition, alkylphosphocholines show selective apoptotic response in tumor cells, whereas normal cells remain unaffected [8,9]. On the basis of imaging and tissue distribution studies in several rodent tumor models, CLR1401 was chosen for follow-up evaluation in human cancer patients. CLR1401 has been tested in a pre-clinical study phase to determine its toxicity and pharmacokinetics in animals. During this study phase, a reliable assay was needed for quantifying CLR1401 in plasma samples. The reliable, validated assay was used to quantify the drug for pre-clinical studies and a similar assay will be used to determine plasma concentrations in clinical trial samples.

The extraction of alkylphosphocholines from plasma samples using protein precipitation [10,11] and solid phase extraction [12,13] has been reported. For the present study, different extraction techniques, including protein precipitation and liquid–liquid extraction, were evaluated to improve extraction efficiency, reduce endogenous interference, and minimize the matrix effect of CLR1401. As a result, 96-well format liquid–liquid extraction using ethyl acetate was developed and used for method validation as well as sample analysis. The method resulted in good recovery, lack of endogenous interference, and moderate matrix effect.

Poor retention, severe peak tailing, and peak broadening of alkylphosphocholines using common reversed-phase columns has been previously reported [10–13]. Only slight peak tailing was observed on a normal-phase column for the analysis of perifosine, an alkylphosphocholine anticancer drug [12]. To achieve good retention and minimize peak tailing of CLR1401, reversed-phase columns and silica columns were evaluated. Among the columns assessed, the Kromasil silica column demonstrated the optimal chromatographic behaviors under hydrophilic interaction

* Corresponding author. Tel.: +1 608 2417229.
E-mail address: Hongliang.Jiang@Covance.com (H. Jiang).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.04.002

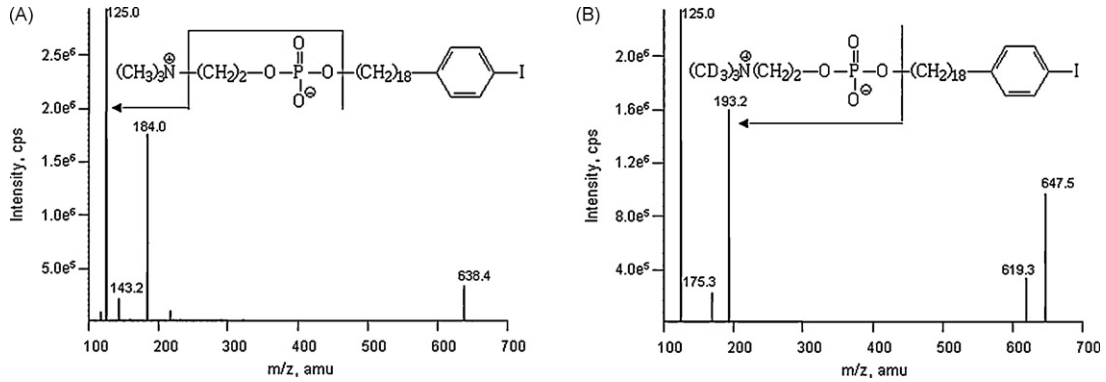

Fig. 1. Representative product ion mass spectra and chemical structure of CLR1401 (A) and CLR1401-d_9 (B), respectively. For mass spectrometer conditions, see Section 2.4.

chromatography, which has been proved to be a powerful tool in bioanalysis of numerous polar compounds when coupled with MS/MS detection [14,15]. The analyte was monitored by electrospray ionization in positive ion multiple reactions monitoring (MRM) mode for the liquid chromatography–tandem mass spectrometry (LC–MS/MS).

The developed method was validated by analyzing three separate batches of rat plasma samples. Subsequently, this validated LC–MS/MS method was used to quantify CLR1401 in incurred rat plasma samples.

2. Experimental

2.1. Chemical, reagents, materials, and apparatus

Ammonium acetate (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, NJ, USA).

HPLC grade acetonitrile and ethyl acetate (EtOAC) were obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade methanol was received from J.T. Baker (Phillipsburg, NJ, USA). PURELAB Ultra system from ELGA (Marlow, UK) was used in the laboratory to produce deionized water. 96-Well flexi-tier block, 2-mL flat bottom glass inserts for 96-well flexi-tier block, and pre-scored molded silicone liner for sealing the glass inserts were obtained from Analytical Sales and Services (Pompton Plains, NJ, USA). Rat plasma with K2-EDTA as the anticoagulant was obtained from Biochemed (Winchester, VA, USA).

An automated SPE system (Quadra 96 model 96-320) for adding organic solvents (ethyl acetate) and transferring samples during sample preparation was obtained from Tomtec (Hamden, CT, USA). A 96-well sample concentrator (SPE DRY-96) with a temperature control from Jones Chromatography (Lakewood, CO, USA) was used for evaporating samples.

2.2. Synthesis of the internal standard CLR1401-d_9

CLR1401 (100% purity) was synthesized according to the published procedure [16]. In the synthesis of internal standard (I.S.) CLR1401-d_9, the method for the introduction of phosphocholine moiety was slightly modified. 18-(*p*-Iodophenyl)-octadecanol was converted to CLR1401-d_9 via the cyclic phosphotriester according to the procedure described in Ref. [17]. The purity of CLR1401-d_9 after chromatographic purification was 99.4%.

2.3. Chromatographic conditions

The HPLC system consisting of solvent delivery system LC-20AD, autosampler SIL-20AC, column oven CTO-20AC, degasser DGU-20A_3, and controller CBM-20A was purchased from Shimadzu (Kyoto, Japan). Chromatographic separation of CLR1401 was evaluated on a Kromasil silica column (50 mm × 3.0 mm, 5 μm) from Thermo Electron (Bellefonte, PA, USA) using hydrophilic interaction liquid chromatography during method development. 20 mM ammonium acetate in water was used as mobile phase A (MA) and pure acetonitrile was used as mobile phase B (MB). For method validation and sample analysis, the chromatographic analysis was conducted under gradient elution. The HPLC program for gradient elution was as follows: 15% of MA (0–0.10 min), from 15% to 30% of MA (0.10–1.20 min), 30% of MA (1.20–2.10 min), from 30% to 15% of MA (2.10–2.20 min), and 15% of MA (2.20–2.80 min). The separation was performed at 1.5 mL/min with column temperature of 25 °C. The sample injection volume was 5 μL.

2.4. Mass spectrometric conditions

An API 5000 triple quadrupole mass spectrometer (MDS-Sciex, Concord, Canada) with turboionspray (TIS) interface was operated in positive ionization mode with multiple reactions monitoring (MRM) for LC–MS/MS analyses. The mass spectrometric parameters were optimized to improve the MRM sensitivity by infusing an approximately 20 ng/mL solution of CLR1401 and CLR1401-d_9 in acetonitrile/water (50/50, v/v) using a Harvard infusion pump (Harvard Apparatus, South Natick, MA, USA). The optimized instrument parameters for monitoring CLR1401 and CLR1401-d_9 were as follows: TIS temperature, 650 °C; TIS voltage, 5500 V; curtain gas (CUR), 30; nebulizing gas (GS1), 50; TIS gas (GS2), 50; collision gas, 5; declustering potential (DP), 190 V; entrance potential (EP), 10 V; collision energy (CE), 32 eV (CLR1401) and 47 eV (CLR1401-d_9); collision cell exit potential (CXP), 16 V. The following precursors to product ion transitions were used for the MRM of CLR1401 and CLR1401-d_9 at m/z 638.4 → 125.0 and m/z 647.5 → 193.2, respectively, with dwell time of 200 ms. Representative product ion mass spectra of these compounds are shown in Fig. 1. The mass spectrometer was operated at unit mass resolution for both Q1 and Q3 quadrupoles.

2.5. Preparation of standard solutions

Stock solutions of CLR1401 at 0.200 mg/mL in methanol were prepared from a preformulated 200 mg/mL solution for the preparation of intermediate solutions. The stock solutions were stored in glass vials and kept refrigerated (2–8 °C). Intermediate standard solutions at the desired concentration for the preparation of calibration curve and QC samples were made by serial dilution. The intermediate internal standard solution (5.00 ng/mL) was diluted from the stock internal standard solution of CLR1401-d_9 at 0.200 mg/mL with methanol–water (50:50, v/v). The internal

standard solutions were stored in glass vials and kept refrigerated (2–8 °C).

2.6. Preparation of calibration standards and quality control samples

Calibration standards were prepared daily by spiking an appropriate quantity of the intermediate standard solutions into pooled rat plasma. Eight calibration standards of CLR1401 were prepared at 2.00, 4.00, 10.0, 20.0, 100, 500, 900, and 1000 ng/mL. Quality control samples were prepared by spiking an appropriate amount of intermediate standard solutions into rat plasma to reach the desired concentration with non-matrix composition less than 5% of the final volume. Lower limit of quantitation QC (LLOQ), low QC (LQC), medium QC (MQC), high QC (HQC), and dilution QC (DQC) were prepared at 2.00, 5.00, 50.0, 750, and 7000 ng/mL, respectively. All QC samples were aliquoted into 1.4 mL polypropylene vials and stored at −20 °C.

2.7. Sample preparation

A volume of 50.0 μL of each calibration standard, QC sample, incurred samples, and blank matrix control sample were aliquoted into individual 2-mL glass tubes sitting in a labeled 96-well format flexi-tier block. Next, 50.0 μL of the intermediate internal standard solution of CLR1401-d_9 at 5.00 ng/mL were added to individual tubes containing samples with the exception of the blank control samples, to which 50.0 μL of methanol–water (50:50, v/v) were added. Then 500 μL of ethyl acetate were added to each sample. Pre-scored silicone liner was used to cover the glass tubes. Vortexing at high speed for approximately 10 min was applied to extract the analyte. The flexi-tier block with samples was centrifuged at 3000 rpm for approximately 5 min. After centrifugation, 250 μL of the organic layer was transferred to a clean 96-well plate using an automated SPE system. The extract was evaporated to dryness using a 96-well sample concentrator (SPE DRY-96) set at 50 °C for about 5 min. The resulting dry residues were reconstituted in 500 μL of acetonitrile–water (85:15, v/v) for LC–MS/MS analysis.

2.8. Data analysis

Sciex Analyst software (version 1.4.1) was used for the data analysis. The calibration curves (analyte peak area/IS peak area for *Y*-axis and analyte concentration/IS concentration for *X*-axis) of CLR1401 were obtained using the least square linear regression fit ($y = mx + b$) and a weighting factor of $1/x^2$. The coefficient of determination (r^2) was set as >0.98 for acceptance criteria of calibration curves. The accuracy and precision were required to be within100 ± 15% of the nominal concentration and ≤15% RSD, respectively, for LQC, MQC, HQC, and DQC samples. The accuracy and precision were required to be within 100 ± 20% of the nominal concentration and ≤20% RSD for LLOQ samples in the intra-batch and inter-batch assay.

2.9. Method validation

CLR1401 was validated over the range of 2.00 ng/mL and 1000 ng/mL for an LC–MS/MS assay. Recovery, matrix effect, specificity, sensitivity, carryover, linearity, precision, accuracy, dilution integrity, and stability were evaluated during method validation. The recovery of extraction for CLR1401 was evaluated by comparing the average absolute peak areas of the analytes extracted from QC samples at the low, medium, and high level with blank plasma that was post-extraction fortified with a neat analyte solution at the same concentration levels. The matrix effect was determined by Matrix Factor (MF), which was obtained as a ratio of the analyte peak response in the presence of matrix ions to the analyte peak response in the absence of matrix ions by post-extraction spiking analyte at MQC level to blank plasma extract and blank water extract. The specificity was assessed by testing 6 lots of blank plasma extract for the presence or absence of interference as well as the lot-to-lot variation regarding interference. Sensitivity of analytes was determined by calculating the signal-to-noise ratio of LLOQ samples. Carryover of analytes was also evaluated by analyzing blank plasma extract samples immediately after an upper limit of quantification (ULOQ) sample or HQC sample. The linearity of the calibration curve was evaluated as described in Section 2.8.

The precision and accuracy of the method were assessed by the analyses of three separate batches of rat plasma samples. Each batch consisted of one set of calibration standards (eight concentration levels) and six replicates of QC samples at each of LLOQ, LQC, MQC, and HQC levels. Dilution integrity was evaluated by a 10-fold dilution of the DQC sample with blank plasma prior to extraction in one of the three batches. The short-term matrix stability was evaluated in one of the three validation batches, in which the LQC and HQC samples were subjected to three freeze–thaw cycles (freeze–thaw stability) or exposed to room temperature (∼22 °C) for approximately 25 h (room temperature stability) prior to extraction. To determine the storage and re-injection reproducibility of the processed samples, one of the three batches of extracted samples was stored in the autosampler (5 °C) for approximately 89 h before re-injection by LC–MS/MS analysis. The long-term stability was evaluated in an additional batch, in which LQC and HQC samples were stored at approximately −20 °C for 397 days. Freshly prepared calibration standards were utilized for each of the stability evaluation.

2.10. Application of the method

The LC–MS/MS method developed for the present study was successfully used for analysis of incurred samples to determine the toxicokinetics of CLR1401 when administered weekly to rats via intravenous injection for 6 weeks. CLR1401 was administered at dose levels of 0.4, 4.0, and 10.0 mg/kg/day to nine toxicokinetic animals/sex/group (groups 6, 7, and 8 respectively). Blood samples were collected from three toxicokinetic animals/sex/group/timepoint on days 1, 22, and 36 at approximately 0.0833, 0.5, 2, 4, 8, and 24 h postdose. Additional blood samples were collected from three toxicokinetic animals/sex/group/timepoint after the day 36 dose at approximately 336, 672, 1008, 1344, 1704, 2016, 2352, 2688, 3024, and 3360 h postdose.

3. Results and discussion

3.1. LC–MS/MS analysis

In previously reported studies, chromatographic separation of alkylphosphocholines, using either reversed- or normal-phase HPLC column, resulted in poor retention or severe peak tailing. The poor chromatography behavior of alkylphosphocholines was likely a result of interaction between the quaternary ammonium group of the phosphocholine molecules and stationary phases of common reversed-phase columns [10,12]. In the current study, reversed-phase columns were also evaluated and yielded poor chromatography during method development (data not shown). Peak shape, for the phosphocholine molecule, was improved when utilizing a silica column under hydrophilic interaction conditions. As shown in Fig. 2, good peak shape and retention was achieved on a Kromasil silica column. This was conducted using a gradient elution

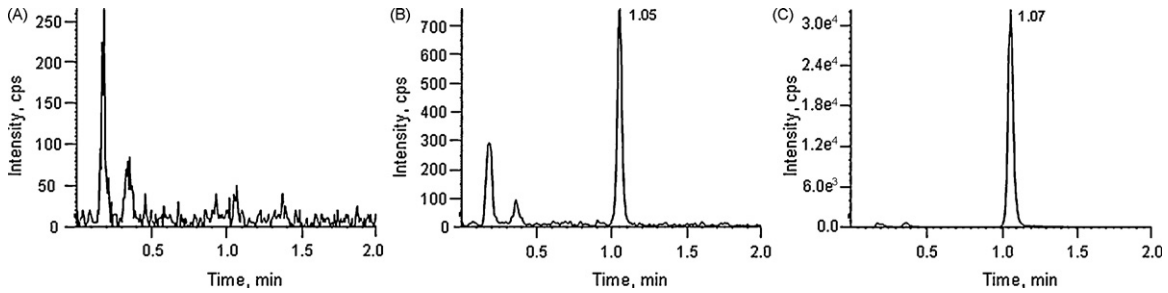

Fig. 2. Representative LC–MS/MS chromatograms of extract from (A) blank plasma; (B) plasma sample spiked with CLR1401 at 2.00 ng/mL (LLOQ); (C) an incurred plasma sample (male, group 8, day 36, hour 8). CLR1401 was monitored at m/z 638.4 → 125.0 in these three chromatograms.

as specified in Section 2.3 after optimizing the mobile phase compositions under isocratic/gradient elution. An HPLC analysis time of 2.8 min was obtained. The relatively short analysis time supported this method for high throughput analysis of incurred samples. No evident endogenous interference from matrix was observed in the chromatographic region of CLR1401 for either method validation or the sample analysis.

For MRM mass spectrometric detection of alkylphosphocholines, two prominent product ions are obtained by cleavage of the alkyl side chain alone or together with further cleavage of the trimethyl amine [12]. Either of these ions can be used to monitor the molecules as shown in Fig. 1. Product ion m/z 125.0 was selected to monitor CLR1401 as a result of its high abundance and the lack of endogenous interference from blank matrix extract. However, for CLR1401-d_9, product ion m/z 193.2 was used for the detection, because significant endogenous interference was observed from blank matrix extract when monitoring m/z 125.0.

3.2. Sample preparation

Based on preliminary data (not shown) obtained during method development, liquid–liquid extraction provided an extract of less interference peaks and matrix effect for the quantification of CLR1401 than protein precipitation. This conclusion was supported by previous studies using liquid–liquid extraction [18]. Therefore, liquid–liquid extraction was selected and evaluated for the method validation and sample analysis. Regarding to liquid–liquid extraction techniques, manual extraction with glass tubes and semi-automated extraction with 96-well plates are traditionally used. However, the manual liquid–liquid extraction with glass tubes is very labor-intensive and time-consuming. Liquid–liquid extraction using 96-well plates may cause potential cross contamination due to the sealing mat of the plate becoming unfixed, as a result of vapor being generated during sample mixing. For the present study, semi-automated liquid–liquid extraction using individual 2-mL glass tubes sitting in a 96-well format flexi-tier block reduced the labor and time as well as minimized the risk of cross contamination by the use of a pre-scored silicone liner to cover the glass tubes, which prevented the spill over of organic solvent but allowed ventilation through a tiny hole in the liner during mixing samples. The total sample preparation time took approximate 45 min. Thus, the optimized sample preparation procedure for the present study was able to be used for high throughput analysis of biological samples.

During method development, ethyl acetate, methyl tert-butyl ether (MTBE), and dichloromethane were evaluated as extraction solvents to improve recovery and minimize the endogenous interference. Compared to MTBE, ethyl acetate showed approximately 3-fold higher recovery for CLR1401, although similar cleanness in extracts was observed. Ethyl acetate demonstrated an approximately 10-fold higher recovery and much cleaner extracts when compared to dichloromethane. As a result, ethyl acetate was selected as the extraction solvent for method validation and sample analysis. The average recovery of the six replicates of QCs at the three levels was 76.2%. The recovery obtained at different concentration levels was also highly consistent (≤2.9% RSD).

3.3. Matrix effect and specificity

The MF of 0.80 and 0.86 was obtained for CLR1401 and CLR1401-d_9, respectively. A MF value of less than one indicates ionization suppression. However, the I.S. normalized MF of 0.93, which is close to one, was obtained as a ratio of the MF of CLR1401 to the MF of CLR1401-d_9. This suggested that matrix effect of CLR1401 can be compensated by its isotope labeled I.S. Therefore, the matrix effect on analysis CLR1401 can be minimal.

Under the current LC–MS/MS and sample preparation conditions, no obvious interference peaks were observed in the chromatographic region of CLR1401 and its internal standard (Fig. 2), suggesting its specificity of this assay.

3.4. Linearity, sensitivity, and carryover

The linearity was assessed based on the average of eight calibrators analyzed in three separate batches. Acceptable linearity was achieved in the range of 2.00–1000 ng/mL. The coefficient of determination (r^2) was greater than 0.997 in all validation batches. The back-calculation results for all calibration standards showed ≤8.1% RSD and −3.8 to 3.0% RE, as summarized in Table 1.

The assay sensitivity was determined by the analysis of LLOQ samples (N = 6) in three separate validation batches. A signal-to-noise ratio (S/N) of approximately 20 was obtained at the LLOQ of 2.00 ng/mL level (shown in Fig. 2). Acceptable precision (6.4% RSD) and accuracy (−6.0% RE) were obtained for inter-day assay (Table 2).

No peaks around the same retention time of CLR1401 were observed in the chromatogram of the blank plasma extract immediately after the ULOQ or HQC sample. As a result, carryover from previous concentrated samples was determined to be negligible.

3.5. Precision and accuracy

The precision and accuracy of the method were determined by analyzing QC samples at the low (5.00 ng/mL, LQC), medium (50.0 ng/mL, MQC), and high (750 ng/mL, HQC) levels. The intra-batch precision was ≤5.9% RSD and the intra-batch accuracy was in the range of −1.4 to −10.8% RE over the three concentration levels evaluated (Table 2). The inter-batch precision and accuracy of QC samples at different levels were also shown in Table 2. These results indicated that excellent precision and accuracy can be achieved for this assay under the current method validation conditions.

Table 1
Accuracy and precision of calibration standards for CLR1401.

	2.00 (ng/mL)	4.00 (ng/mL)	10.0 (ng/mL)	20.0 (ng/mL)	100 (ng/mL)	500 (ng/mL)	900 (ng/mL)	1000 (ng/mL)
N	3	3	3	3	3	3	3	3
Mean	1.98	4.01	10.3	20.4	96.2	501	900	992
RSD (%)	4.1	8.1	0.6	3.9	5.0	0.8	1.7	1.3
RE (%)	−1.0	0.3	3.0	2.0	−3.8	0.2	0.0	−0.8

Table 2
Precision and accuracy of quality control samples of CLR1401.

	LLOQ (2.00 ng/mL)	LQC (5.00 ng/mL)	MQC (50.0 ng/mL)	HQC (750 ng/mL)	DQC (7000 ng/mL)
Day 1					
N	6	6	6	6	6
Mean	1.90	4.90	49.3	694	6260
RSD (%)	4.2	3.9	2.2	0.6	5.5
RE (%)	−5.0	−2.0	−1.4	−7.5	−10.6
Day 2					
N	6	6	6	6	
Mean	1.96	4.88	48.9	669	
RSD (%)	5.8	5.5	1.7	5.9	
RE (%)	−2.0	−2.4	−2.2	−10.8	
Day 3					
N	6	6	6	6	
Mean	1.78	4.90	49.0	687	
RSD (%)	5.4	4.0	2.3	1.5	
RE (%)	−11.0	−2.0	−2.0	−8.4	
Inter-day					
N	18	18	18	18	
Mean	1.88	4.90	49.1	683	
RSD (%)	6.4	4.3	2.0	3.7	
RE (%)	−6.0	−2.0	−1.8	−8.9	

Note: A 10-fold dilution of the DQC with blank matrix prior to extraction was applied.

Table 3
Freeze/thaw stability, room temperature stability, re-injection reproducibility, and long-term stability of CLR1401.

	LQC (5.00 ng/mL)	HQC (750 ng/mL)
Stability after three freeze–thaw cycles, *N* = 6		
Mean	4.72	695
RSD (%)	4.3	0.8
RE (%)	−5.6	−7.3
Room temperature stability for 25 h, *N* = 6		
Mean	4.73	701
RSD (%)	3.6	3.0
RE (%)	−5.4	−6.5
Re-injection reproducibility for 89 h, *N* = 6		
Mean	4.80	655
RSD (%)	5.4	6.0
RE (%)	−4.0	−12.7
Storage at −20 °C for 397 days, *N* = 6		
Mean	5.13	796
RSD (%)	6.0	2.3
RE (%)	2.6	6.1

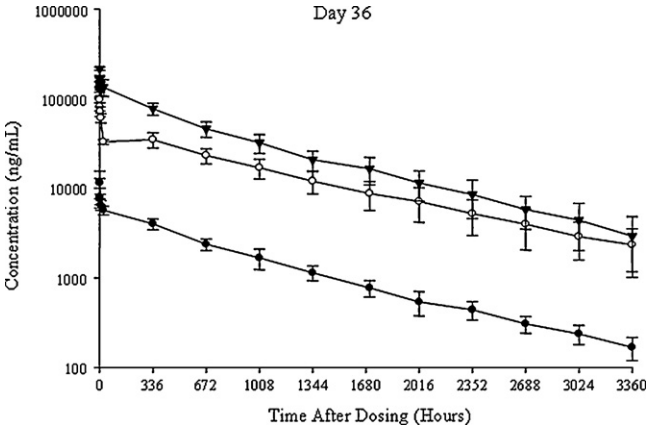

Fig. 3. Mean concentration (ng/mL) of CLR1401 in combined male and female rat plasma after intravenous administration of CLR1401 at different doses: (●) represents dose group 6 at 0.4 mg/kg/day; (○) represents dose group 7 at 4.0 mg/kg/day; (▼) represents dose group 8 at 10.0 mg/kg/day. *Note*: error bars (I) represent standard deviation.

3.6. Dilution integrity

In order to evaluate sample dilution integrity at a concentration higher than ULOQ, the dilution QC samples (DQCs) were subjected a 10-fold dilution with blank matrix prior to extraction. As shown in Table 2, the results demonstrated that samples with a concentration greater than the upper limit of the standard curve could be quantified with reliable precision (5.5% RSD) and accuracy (−10.6% RE) after being appropriately diluted with blank matrix.

3.7. Stability

To evaluate the short-term stability of incurred samples during shipment, short-term storage, and sample preparation process, experiments were designed and conducted under different conditions including freeze–thaw cycle analysis and room temperature exposure analysis using LQC and HQC samples. To evaluate post-processing stability, one batch of extracted samples was investigated after short-term storage at refrigerated conditions. In addition, LQC and HQC samples were also used to assess long-term stability at −20 °C storage condition. No stability issue was observed from any of these experiments, referring to the data summarized in Table 3 where all necessary information is clearly presented.

3.8. Application of the method

A representative chromatogram of an incurred sample (male, group 8, day 36, hour 8) is shown in Fig. 2, which indicates similar chromatographic behavior to QCs. More than 600 plasma samples collected incurred samples were analyzed so far and raised no problems during quantification of CLR1401.

Mean plasma concentration–time profiles of the sexes combined on day 36 are presented in Fig. 3. As shown in Fig. 3, the analyte was being slowly eliminated due to the nature of the compound. Day 36 toxicokinetic data with recovery is shown as an example to characterize the complete toxicokinetic profile. As indicated, exposure to CLR1401 increased with the increase in dose level from 0.4 to 10.0 mg/kg/day. In summary, CLR1401 was slowly eliminated and moderately distributed in rats following intravenous injection.

4. Conclusion

A rapid, specific, and reliable LC–MS/MS based bioanalytical method has been successfully developed and validated to quantify CLR1401 in rat plasma. The current chromatographic conditions provide both good retention and peak shape for the analysis of CLR1401, which set up a successful example of analyzing alkylphosphocholine under hydrophilic interaction chromatography. In addition, 96-well format liquid–liquid extraction with ethyl acetate results in clean samples with high recovery. The relatively short sample preparation time together with the short LC run time make the present method more practical for high throughput sample analysis. The present assay demonstrates highly reproducible chromatographic and statistical results in terms of precision and accuracy during method validation. The successful application of this method to a toxicokinetic study further supports its applications in future pharmacokinetic study of clinical samples.

Acknowledgements

The authors thank Xuemin Jiang, Peter Sausen, Tom Addison, and Kevin Jones at Covance Laboratories, Inc., Madison, Wisconsin, for their help and support in the current project.

References

[1] C. Oberle, U. Massing, H.F. Krug, Biol. Chem. 386 (2005) 237.
[2] S.R. Vink, J.H.M. Schellens, W.J. van Blitterswijk, M. Verheij, Invest. New Drugs 23 (2005) 279.
[3] U. Naumann, J. Wischhusen, S. Weit, J. Rieger, H. Wolburg, U. Massing, M. Weller, Cell Death Differ. 11 (2004) 1326.
[4] C. Belka, V. Jendrossek, M. Pruschy, S. Vink, M. Verheij, W. Budach, Int. J. Radiat. Oncol. Biol. Phys. 58 (2004) 542.
[5] M. Agresta, P. D'Arrigo, E. Fasoli, D. Losi, G. Pedrocchi-Fantoni, S. Riva, S. Servi, D. Tessaro, Chem. Phys. Lipids 126 (2003) 201.
[6] J. Kotting, M.R. Berger, C. Unger, H. Eibl, Cancer Chemother. Pharmacol. 30 (1992) 105.
[7] N. Marschner, J. Kotting, H. Eibl, C. Unger, Cancer Chemother. Pharmacol. 31 (1992) 18.
[8] F. Mollinedo, J.L. Fernandez-Luna, C. Gajate, B. Martin-Martin, A. Benito, R. Martinez-Dalmau, M. Modolell, Cancer Res. 57 (1997) 1320.
[9] C. Gajate, F. Mollinedo, Curr. Drug Metab. 3 (2002) 491.
[10] E.W. Woo, R. Messmann, E.A. Sausville, W.D. Figg, J. Chromatogr. B 759 (2001) 247.
[11] L.H. Lindner, H. Eibl, M. Hossann, M. Vogeser, J. Chromatogr. B 869 (2008) 16.
[12] N.G. Knebel, S. Grieb, M. Winkler, M. Locher, E. Van der Vlis, E.R. Verheij, J. Chromatogr. B 721 (1999) 257.
[13] T.P.C. Dorlo, M.J.X. Hillebrand, H. Rosing, T.A. Eggelte, P.J. de Vries, J.H. Beijnen, J. Chromatogr. B 865 (2008) 55.
[14] A. Eerkes, W.Z. Shou, N. Weng, J. Pharm. Biomed. Anal. 31 (2003) 917.
[15] W. Li, Y. Li, D.T. Francisco, N. Weng, Biomed. Chromatogr. 19 (2005) 385.
[16] A.N. Pinchuk, M.A. Rampy, M.A. Longino, R.W.S. Skinner, M.D. Gross, J.P. Weichert, R.E. Counsell, J. Med. Chem. 49 (2006) 2155.
[17] U.F. Heiser, B. Dobner, J. Chem. Soc., Perkin Trans. 1 (1997) 809.
[18] E. Chambers, D.M. Wagrowski, Z. Lu, J.R. Mazzeo, J. Chromatogr. B 852 (2007) 22.