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Liquid chromatography-tandem mass spectrometric assay for the quantitative determination of the tyrosine kinase inhibitor quizartinib in mouse plasma using salting-out liquid-liquid extraction

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ABSTRACT

A bioanalytical assay for quizartinib -a potent, and selective FLT3 tyrosine kinase inhibitor- in mouse plasma was developed and validated. Salting-out assisted liquid-liquid extraction (SALLE), using acetonitrile and magnesium sulfate, was selected as sample pretreatment with deuterated quizartinib as internal standard. Separation was performed with reversed-phase liquid chromatography followed by detection with positive electrospray-triple quadrupole mass spectrometry in the selected reaction monitoring mode. The assay was successfully validated for mouse plasma in a 2–2000 ng/ml calibration range with r^2 = 0.9958 ± 0.0028 (n = 7) for linear regression with the inverse square of the concentration as a weighting factor. The within-run precision (n = 18), between-run precision and accuracy were 2.9–6.0%, 4.5–8.9% and 91.7–109.4% respectively. The drug was stable under all relevant conditions. Finally, the assay was successfully applied in a pharmacokinetic pilot study in plasma of FVB/NRj mice treated with quizartinb orally.

1. Introduction

Quizartinib (AC220, Fig. 1) is an fibromyalgia syndrome-like tyrosine kinase 3 (FLT3) inhibitor under development for the treatment of patients with acute myeloid leukemia (AML). This drug has been developed in an oral dosage form and is being clinically tested in a phase III trial. The trial is expected to be completed in the year 2018 [1]. FLT3, a class III tyrosine kinase receptor, is essential for stem cell development and the immune system, while the ligands are produced by bone marrow and other cells. In a recent review, FLT3 expression is reported to be found at a high level of 70–100% in AML patients and has been indicated to have a role in the proliferation of leukemia blasts [2]. It has also been shown that approximately 30% of the AML patients have mutations in FLT3 as somatic alteration [2,3]. The most frequent mutation that occurred was an internal tandem duplication (ITD) [2,3] at around 24% [2]. Quizartinib is proven selective for FLT3 and can inhibit both ITD and wild type FLT3 in cell line models [4]. Another in vitro study describes that quizartinib could, dependent on the mutation, also inhibit cellular proliferation of platelet-derived growth factor receptor (PDGF-R) and kinase receptor (KIT), two other tyrosine kinase receptors that are often found mutated in leukemia [5], making this drug even more a promising candidate for further clinical research.

A phase I study has shown that the highest human plasma concentration from a 200 mg daily oral dose was approximately 1000 ng/ml. Quizartinib also showed a long half-life, estimated of more than 1.5 days based on the same study [6]. A mass balance study of a single

Abbreviations: FLT3, FMS like tyrosine kinase 3; SALLE, salting-out liquid-liquid extraction; AML, acute myeloid leukemia; ITD, internal tandem duplication; KIT, kinase receptor; PDGF-R, platelet derived growth factor receptor; IS, internal standard; QC, quality control; LLoQ, lower limit of quantification; EMA, European Medicine Agency; AUC, area under the plasma concentration-time curve; ESI, electrospray ionization

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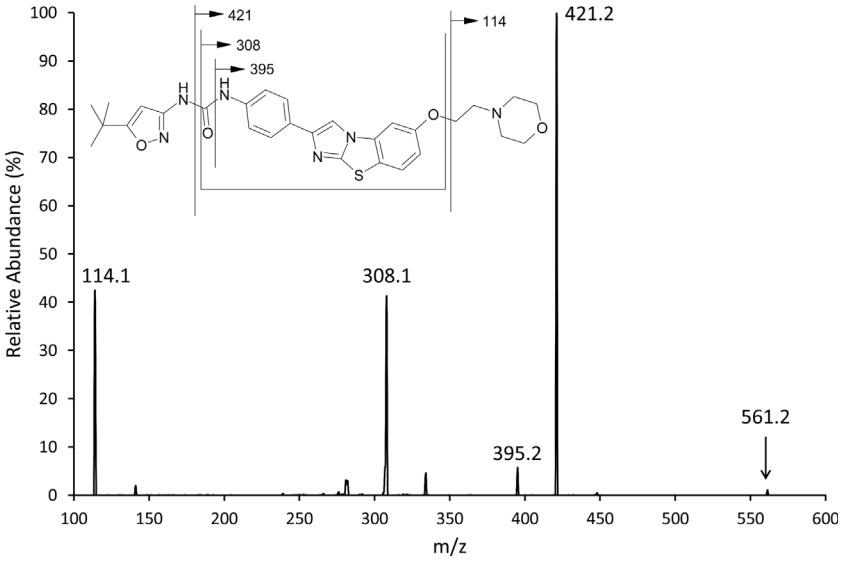

Fig. 1. Chemical structure of quizartinib and the product spectrum, formed by collision induced dissociation of the protonated molecule of quizartinib; *m/z* 561.2@–35 V. Dissociation pathways of the reaction have been proposed.

dose of quizartinib (60 mg, with 1.6% ^{14}C-quizartinib) in 6 healthy men has revealed that (radioactive) quizartinib was rapidly and highly orally available with a maximum blood concentration (296 ± 67 ng equivalents/g, equivalent to 311 ± 70 ng/ml non-labeled quizartinib based on a blood density of 1.05 g/mL) achieved 4 h after dosing [7,8]. It also has indicated that quizartinib was primarily eliminated in the feces (76.3 ± 6.2% of total radioactivity). According to the same experiment, the primary metabolite of quizartinib in the circulation is AC886 which is its mono-oxidative form with hydroxylation at the *tert*-butyl group [8].

The pharmacokinetic profiles of quizartinib in mice [4] and rats [9] have been reported using LC–MS/MS as the bioanalytical method, but hardly any details of the assays have been divulged yet. Other bioanalytical methods have not been published hitherto. Therefore, we developed and validated an LC–MS/MS assay employing a small volume of mouse plasma (10 µl) for pharmacokinetic studies of quizartinib in mice using a fast, simple, and an efficient salt-assisted liquid-liquid extraction (SALLE) as sample pretreatment.

2. Material and methods

2.1. Chemicals and reagents

Quizartinib (98.5%, M_w = 560.7 g/mol) was obtained from Sequoia Research Products (Pangbourne, UK) and [$^2H_4$]-labeled quizartinib, (100%, M_w = 564.7 g/mol) was obtained from Alsachim (Strasbourg, France). Water (LC–MS grade), methanol (HPLC grade), acetonitrile (HPLC-S grade) were acquired from Biosolve (Valkenswaard, The Netherlands). Water, not used as eluent, was home purified by reverse of osmosis on a multi-laboratory scale. Analytical grade of ammonium hydroxide and magnesium sulfate were supplied by Sigma-Aldrich (Steinheim, Germany). Lithium Heparin female mouse plasma and pooled human plasma were obtained from Seralab (West Sussex, UK).

2.2. Equipment

The LC–MS/MS instrument consisted of an Accela pump and autosampler, and a TSQ Quantum Ultra quadrupole mass spectrometer with heated electrospray ionization (Thermo Fischer Scientific, San Jose, CA, USA). The equipment was controlled by Thermo Fischer Xcalibur software (version 2.07). This software was also used to record and process all the data.

2.3. LC–MS/MS conditions

The injection (2 µl partial loop) was made on an Aquity UPLC® BEH C18 column (30 × 2.1 mm, d_p = 1.7 µm, Waters, Milford, USA) protected by UPLC® BEH C18 VanGuard pre-column (5 × 2.1 mm, d_p = 1.7 µm, Waters). The temperature of column and auto-sampler were maintained at 40 °C and 4 °C respectively during the analytical run. The mobile phase was a mixture of methanol, water, and ammonium hydroxide 2% (v/v) with proportions 70:25:5 (v/v). Isocratic elution was used at 0.6 ml/min during 1 min. Then, the column was flushed with 100% acetonitrile for 0.2 min and finally, the column was reconditioned at the isocratic conditions for 0.8 min until starting the next injection. The whole eluate was transferred into the electrospray probe, starting from 0.5 min until 1.4 min after injection by switching the MS divert valve. The electrospray was tuned in the positive ionization mode by introducing 5 µl/min of 5 µg/ml quizartinib together with 0.6 ml/min of a solvent mixture containing 50% (v/v) of methanol and 50% (v/v) of 0.1% (v/v) formic acid in water. A 3000 V spray voltage with 358 °C vaporizer and 336 °C capillary temperatures were used in the positive ionization mode. The nitrogen sheath, ion sweep, and auxiliary gasses were set at 60, 4, and 15 arbitrary units respectively. The skimmer voltage was −10 V and the SRM mode was using argon as collision gas at 1.3 mTorr. The peak resolutions were set at 0.7 full widths at half height (unit resolution) for both separating quadrupoles. The tube lens off set was 131 V for all the transitions. Quizartinib was monitored at *m/z* 561.2 → 114.1; 308.1; 421.2 at −41, −40, −26 V collision energies respectively, [$^2H_4$]-quizartinib was monitored at *m/z* 565.2 → 425.2 at −26 V collision energy.

2.4. Standard and quality control solutions

A 500,000 ng/ml quizartinib stock solution was prepared by weighing 200–400 µg of quizartinib and dissolving it in methanol. This stock solution was further diluted with 50% (v/v) methanol in water to obtain a working solution at a concentration of 100,000 ng/ml. The highest calibration solution (2000 ng/ml) was prepared by diluting the working solution in pooled blank female mouse plasma and was stored in polypropylene tubes at −30 °C until further use. A 200,000 ng/ml [$^2H_4$]-quizartinib (IS) stock solution was prepared in methanol. The IS stock solution was diluted further to a concentration of 50 ng/ml in acetonitrile for daily use and was also stored at −30 °C.

A second set of a 500,000 ng/ml quizartinib stock solution and a 100,000 ng/ml working solution was prepared and further diluted in blank female mouse plasma to obtain 1600 (QC-high), 80 (QC-med), 4

(QC-low) and 2 (QC-LLoQ) ng/ml quality control (QC) samples. The QC solutions were stored in the same way as the highest calibration solution; the working solution was freshly prepared when used.

2.5. Sample preparation

In a 0.5-ml micro polypropylene tube, 25 µl of 50 ng/ml [$^2H_4$]-quizartinib (IS) in acetonitrile and 5 µl of 3 M $MgSO_4$ were added to a 10-µl mouse plasma sample. The sample was vortex mixed vigorously for 15 s followed by centrifugation at 10,000 × *g* for 2 min at 20 °C. After centrifugation, 20 µl of clear supernatant was transferred into a glass injection vial with a 250 µl glass insert and supplemented with 100 µl of 25% (v/v) methanol in water. After vortex mixing the closed vial briefly, the sample was ready for injection.

2.6. Analytical method validation

International guidelines were used as the laboratory framework for these validation procedures [10,11].

2.6.1. Calibration

The quizartinib calibration samples were prepared by serial dilution of the highest calibration solution (2000 ng/ml quizartinib in mouse plasma) to 1000, 200, 100, 20, 10, and 2 ng/ml in blank pooled female mouse plasma. These samples were prepared in duplicate (n = 14) for each daily use. Least-squares linear regression with reversed squared of the concentration ($1/x^2$) as a weighting factor was employed to define the calibration curve using the ratio of the analyte (quizartinib) and IS ([$^2H_4$]-quizartinib) peak areas.

2.6.2. Precision and accuracy

The QC-high (1600 ng/ml), -medium (80 ng/ml), -low (4 ng/ml), and -LLoQ (2 ng/ml) samples were used to assess the precision and accuracy of the assay. Precision and accuracy were determined by performing sextuple analysis of each QC level on three separate days for all QCs (total: n = 18 per QC). Relative standard deviations were calculated for both, the within- and between-day precisions. In addition, the dilution integrity of the mouse plasma samples (1600 ng/ml) diluted 5 times with pooled human lithium heparin plasma was investigated (n = 6).

2.6.3. Selectivity

To assess the assay selectivity, six individual lithium heparin female mouse plasma samples were processed. The samples were prepared without quizartinib and IS (double blank samples), without quizartinib (blank samples) and with quizartinib at the LLoQ level (2 ng/ml of quizartinib), supplemented with the IS. The response of quizartinib and IS in the blank samples was compared with the quizartinib LLoQ response.

2.6.4. Recovery and matrix effect

The recovery was determined (n = 5) by comparing processed samples (QC-high, -medium, and -low) with the reference solutions of quizartinib in blank mouse plasma extract at the same levels. The matrix effect was determined by two methods. The absolute matrix effect was assessed utilizing a post-column infusion method. A solution of 10 µg/ml quizartinib and 10 µg/ml IS in 25% methanol was introduced post-column at 5 µl/min together with the eluate at 600 µl/min while the individual double blank plasma samples (without quizartinib and IS, n = 6) were injected onto the column without using the divert valve. The inter-lot relative matrix effect was assessed by processing individual mouse plasma at the QC-high and -low levels (n = 6 for each level) followed by comparison to the reference solution without matrix at the same level. The IS-normalized matrix factors were calculated based on the EMA guideline [10].

2.6.5. Stability

The stability of quizartinib was investigated in the QC-high and -low plasma samples. Quadruplicate analysis of these samples from separate tubes was performed after storage at ambient temperature (± 20 °C) for 24 h, at −30 °C interrupted by three freeze-thaw cycles (thawing at 20 °C for ± 1 h, and freezing again at least for one day at −30 °C), and after 2 months at −30 °C. In addition, an analytical run was reinjected after additional storage of the extracts at 4 °C for 3, 7 and 14 days for testing the stability of the post-preparative samples in the auto-injector.

Finally, the responses of quizartinib from the stock solution in methanol after 6 h at room temperature and 2 months at −30 °C (n = 2) were compared to freshly prepared stock solutions with LC–MS/MS after appropriate dilution and adding IS.

2.7. Pharmacokinetic study in mice

For the pharmacokinetic pilot study, female mice (n = 4) with a FVB/NRj genetic background (wild type) were fasted for 3 h and then received quizartinib (10 mg/kg) orally. Quizartinib was dissolved in DMSO at a concentration of 33.3 mg/ml, and diluted with 50 mM sodium acetate (pH 4.4) to yield a concentration of 1 mg/ml for the administered working solution, using 10 ml/kg body weight. Working solutions were prepared freshly on the day of the experiment. Blood samples were collected in lithium heparin-containing microvettes from the tail vein at 0.5, 1, 2, 4, and 8 h after administration. Mice were anesthetized with isoflurane 24 h after administration, and the final blood samples were collected by cardiac puncture. After centrifugation at 9000 × *g* for 6 min at 4 °C, plasma samples were stored at −30 °C prior to analysis. The mouse plasma samples were diluted 5 times with human lithium heparin plasma. Ten µl of this diluted sample was treated as reported in Section 2.5.

Pharmacokinetic parameters were calculated, including area under plasma concentration-time curve ($AUC_{0 \rightarrow 24}$), time to reach maximum plasma concentration (t_{max}) and maximum plasma concentration (C_{max}). The $AUC_{0 \rightarrow 24}$ was calculated using the trapezoidal rule.

Incurred samples reanalysis was conducted 4 months after the first analysis. Twenty diluted samples were investigated for this parameter [10].

3. Results and discussion

3.1. Method development

For analytical method development, the AC886 metabolite was not incorporated in the bioanalytical assay because it was not commercially available. In ESI, quizartinib showed two responses of a protonated form, a single protonated (*m/z* = 561.2) and double protonated (*m/z* 281.2) molecule. Positive ESI–MS/MS settings were optimized for single protonated quizartinib (*m/z* 561.2) to obtain maximum sensitivity. A product spectrum of the single protonated quizartinib is shown in Fig. 1.

The chromatographic method was optimized empirically, based on MS response, retention time, and peak shape. Ammonium hydroxide showed a higher response of quizartinib than formic acid when used in the mobile phase. In addition, it also reduced the formation of the double protonated ion. Moreover, these alkaline conditions resulted in a narrower chromatographic peak. The final combination of methanol, water and ammonium hydroxide showed satisfactory chromatographic properties with a 2 min total run time and with quizartinib and IS eluting within 1 min (Fig. 2). The acetonitrile flush for 0.2 min after elution of the analyte was used to ensure the removal of strongly retained plasma constituents from the column. All the optimum ESI parameters were reconfirmed in the finally selected eluent after chromatographic optimization.

The chosen sample preparation method for this assay was SALLE. For SALLE, acetonitrile is by far the most frequently applied extraction

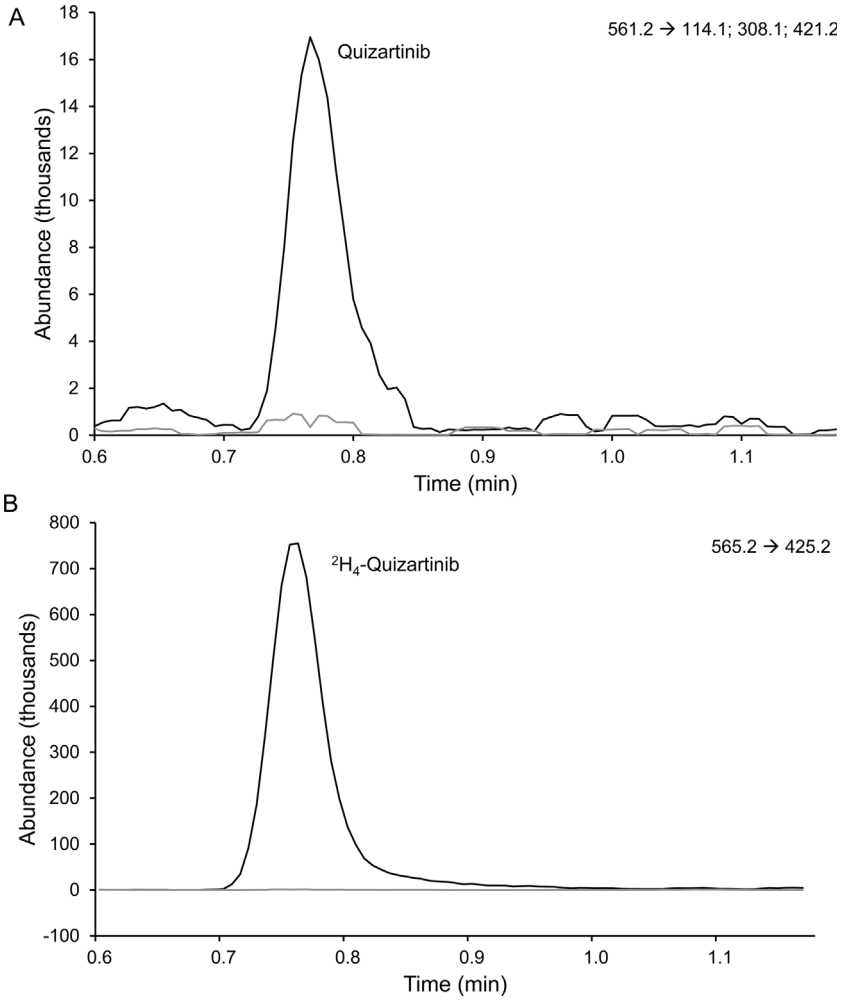

Fig. 2. Chromatograms of (A) quizartinib and (B) IS ($^2H_4$-quizartinib) in mouse plasma at the LLoQ level of quizartinib (2 ng/ml) compared to a double blank plasma sample (in grey).

solvent, for sufficient phase separation a solvent ratio (organic to aqueous) of at least 1.5 is required, for optimal recovery a higher ratio is often necessary. Magnesium sulfate was selected as salt for SALLE for its high recovery and precision with a clear visible phase separation. $MgSO_4$ enabled a precipitated layer forming in between two phases after centrifugation. This layer gave visual aid in pipetting off the supernatant [12].

3.2. Analytical method validation

3.2.1. Calibration

The relative response of quizartinib showed a linear trend over the investigated range. The average of the regression parameters of the linear regression function for 7 runs was $Y = 0.0077\ (\pm 0.0041) + 0.0163\ (\pm 0.0029) \times X$ with a regression coefficient of 0.9958 (± 0.0028) using the inverse square of the concentration as a weighting factor. *X* denotes the quizartinib concentration (ng/ml) while *Y* denotes the drug response relative to the IS response. For the seven calibrations above, the concentrations were back-calculated (data not shown) from the ratio of the peak area (quizartinib and IS) in which they were included. There was not any average deviation higher than 5.8% of the nominal value for each level, indicating the method was sufficiently capable of providing a linear calibration [10,11].

3.2.2. Precision and accuracy

The accuracy and precision data of the method at four different concentrations are shown in Table 1. The within-day precision, between-day precision, and accuracy were within ± 15% for QC-high, -medium and -low and for QC-LLoQ they were within ± 20%. Therefore, these results fulfilled the guideline requirements [10,11]. For dilution integrity (5 times dilution of 1600 ng/ml quizartinib, n = 6) 105.9% and 7.79% were found for accuracy and within-day precision respectively. Both results were within ± 15%; therefore, 5 times dilution of the very small mouse plasma samples with human plasma could be used for the pharmacokinetic pilot study.

Table 1
Precision and accuracy data of the quizartinib assay. The data were obtained from three analytical runs in three separate days (n = 18).

Nominal concentration (ng/ml)	Within-run precision (%)	Between-run precision (%)	Between-run accuracy (%)
1600	4.3	6.3	91.7
80	2.9	4.5	98.8
4	6.0	8.0	104.5
2	6.8	8.9	109.4

3.2.3. Selectivity

The analysis of six independent blank (without quizartinib) and double blank (without quizartinib and IS) mouse plasma samples showed no interferences of quizartinib and labeled IS respectively. The responses of blank samples were 5.8 ± 4.0% of the LLoQ responses for quizartinib while the double blank responses were 0.11 ± 0.07% of the IS responses, meeting the required maximum responses of 20% and 5% for the quizartinib and IS [10]. The LLoQ (2 ng/ml, n = 6) signals were higher than five times the blank response as shown in Fig. 2A, and

Table 2
Stability data of mouse plasma samples (mean of % recovery ± SD) after various storage condition.

Storage condition	QC-high (1600 ng/ml)	QC-low (4 ng/ml)
24 h at ambient temperature	86.5 ± 3.1	105.1 ± 5.4
3 freeze-thaw cycles	94.7 ± 5.5	106.5 ± 2.8
2 months at −30 °C	99.2 ± 4.6	88.9 ± 3.1

corresponded to 1.8 ± 0.1 ng/ml. These findings indicate that this method is able to quantify quizartinib at the LLoQ level [10,11].

3.2.4. Recovery and matrix effect

The extraction recoveries for QC-high, -medium, and -low samples were 79.2 ± 2.0%, 79.1 ± 10.1%, and 83.8 ± 7.8% respectively, confirming their consistency, precision, and reproducibility [11]. Utilizing the post-column infusion method for inter-lot blank plasma samples, no matrix effect was observed during a 0.4–1.0 min retention time window (n = 6, data not shown). The inter-lot IS normalized matrix factors were 97.5 ± 4.4% and 97.5 ± 14.4% for QC-high and -low levels. The inter-lot relative matrix factor variation of the QC-high and -low levels both were below 15%, as required by the EMA guideline [10]. These results denoted the absence of a significant matrix effect [13] in the assay.

3.2.5. Stability

The stability data of quizartinib in lithium heparin female mouse plasma after different storage conditions are presented in Table 2. Quizartinib demonstrated sufficient stability (variations and accuracies within ± 15% [10]) under all investigated storage conditions.

Re-injection of the post-preparative calibration and QC samples after additional storage for 3, 7, and 14 days at the 4 °C, respectively, resulted again in successful analysis without loss of accuracy and precision. The number of QC failures was 2 or 3 out of 24 samples in all analytical runs. Therefore, QC failures remained far below a 33% frequency as required during at least two weeks [10]. Finally, the stock stability after a 6 h exposure to room temperature and after 2 months storage at −30 °C (n = 2) resulted in recovery data for both experiments within ± 5% (99.3 and 103.3% respectively). These data indicated that stock solutions can be used at least up to 2 months after storage at −30 °C.

Table 3
Incurred sample reanalysis of 20 diluted plasma samples after storage at −30 °C for 4 months.

Initial concentration (ng/ml)	Incurred reanalysis concentration (ng/ml)	Difference (%)
255.7	253.7	−0.8
432.4	380.0	−12.9
398.5	424.1	6.2
708.6	694.3	−2.0
2011.6	2134.7	5.9
155.7	124.4	−22.3
593.1	620.1	4.4
2544.1	2704.7	6.1
452.9	442.0	−2.4
807.4	779.9	−3.5
142.5	159.1	11.0
217.9	224.8	3.1
446.7	446.5	0.0
656.5	614.5	−6.6
1574.0	1551.3	−1.5
229.2	207.1	−10.1
562.2	524.2	−7.0
326.5	275.0	−17.1
1607.2	1467.5	−9.1
274.0	244.0	−11.6

3.3. Pharmacokinetic study in mouse plasma

The profile of quizartinib concentration versus time in wild-type FVB/NRj mice is illustrated in Fig. 3. The graph shows that quizartinib had a slow absorption illustrated by the increasing level of quizartinib until 8 h after administration.

Pharmacokinetic parameters, C_{max} (2018 ± 386 ng/ml), t_{max} (8 h), and $AUC_{0\rightarrow 24}$ (26,648 ± 6033 ng h/ml) were calculated (n = 4). Elimination phase related parameters, $t_{1/2}$, K_e, and K_a, were not calculated because the obtained data were insufficient (only 2 data points, i.e., 8 and 24 h after administration). Thus, for assessing the elimination and absorption parameters of quizartinib an extended study in this mouse strain will be needed. In NU/NU mice similar C_{max} values have been observed [4]; however, all other parameters seem significantly different with slower absorption, slower elimination and higher exposure in the wild-type FVB/NRj mice.

Finally, incurred samples (n = 20) were reanalyzed after storage at −30 °C for 4 months to investigate the interference of known and unknown metabolites that might affect the reproducibility [14]. The results (Table 3) show that only one replication had a difference larger

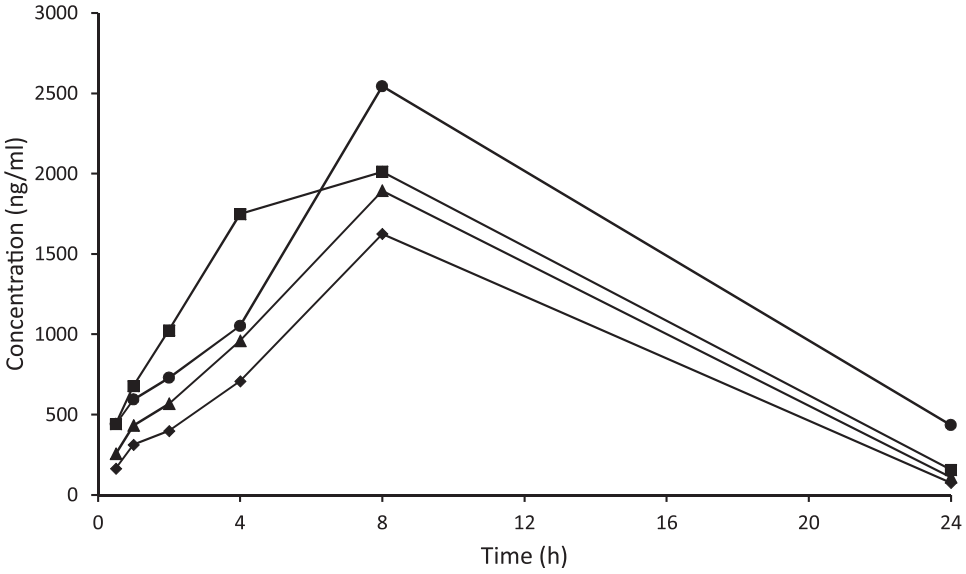

Fig. 3. Pharmacokinetic plots for four studied FVB/NRj mice, treated orally with 10 mg/kg quizartinib.

than 20%. This finding fulfilled the guideline criteria [10] and implied that there was no intervention of a known or unknown metabolite that affected the drug quantification.

4. Conclusions

Our results show that the values of accuracy, precision, and stability fulfilled international guidelines [10,11]. The assay was successfully validated and applied to measure quizartinib in mouse plasma. The present pharmacokinetic pilot study indicated that quizartinib has a slow absorption rate. Further research should be conducted to determine the elimination rate of quizartinib more precisely to obtain a full pharmacokinetic profile of quizartinib. The presented LC–MS/MS assay with SALLE produced reliable and reproducible quantitative data of quizartinib so it can be used for future pharmacokinetic studies with this drug.

References

[1] (QuANTUM-R). An Open-label Study of Quizartinib Monotherapy vs. Salvage Chemotherapy in Acute Myeloid Leukemia (AML) Subjects Who Are FLT3-ITD Positive, (n.d.). https://clinicaltrials.gov/ct2/show/NCT02039726 (Accessed 12 January 2017).
[2] D. Gary Gilliland, J.D. Griffin, The roles of FLT3 in hematopoiesis and leukemia, Blood 100 (2002) 1532–1542.
[3] D. Small, FLT3 mutations: biology and treatment, Hematology Am. Soc. Hematol. Educ. Program (2006) 178–184.
[4] P.P. Zarrinkar, R.N. Gunawardane, M.D. Cramer, M.F. Gardner, D. Brigham, B. Belli, M.W. Karaman, K.W. Pratz, G. Pallares, Q. Chao, K.G. Sprankle, H.K. Patel, M. Levis, R.C. Armstrong, J. James, S.S. Bhagwat, AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML), Blood 114 (2009) 2984–2992.
[5] K.M. Kampa-Schittenhelm, M.C. Heinrich, F. Akmut, H. Döhner, K. Döhner, M.M. Schittenhelm, Quizartinib (AC220) is a potent second generation class III tyrosine kinase inhibitor that displays a distinct inhibition profile against mutant-FLT3, -PDGFRA and -KIT isoforms, Mol. Cancer 12 (2013) 19.
[6] J.E. Cortes, H. Kantarjian, J.M. Foran, D. Ghirdaladze, M. Zodelava, G. Borthakur, G. Gammon, D. Trone, R.C. Armstrong, J. James, M. Levis, Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukemia irrespective of FMS-like tyrosine kinase 3-internal tandem duplication status, J. Clin. Oncol. 31 (2013) 3681–3687.
[7] J. Li, G. Bresnahan, G. Gammon, M. Sanga, C. Hale, T. Hashimoto, S. Gill, J. James, Absorption, metabolism, and excretion of quizartinib (AC220), a FLT3 tyrosine kinase inhibitor for treatment of acute myeloid leukemia, in healthy male volunteers, Blood 120 (2015) 4327.
[8] M. Sanga, J. James, J. Marini, G. Gammon, C. Hale, J. Li, An open-label, single-dose, phase I study of the absorption, metabolism, and excretion of quizartinib, a highly selective and potent FLT3 tyrosine kinase inhibitor, in healthy male subjects, for the treatment of acute myeloid leukemia, Xenobiotica (2016) 1–43.
[9] Q. Chao, K.G. Sprankle, R.M. Grotzfeld, A.G. Lai, T.A. Carter, A.M. Velasco, R.N. Gunawardane, M.D. Cramer, M.F. Gardner, J. James, P.P. Zarrinkar, H.K. Patel, S.S. Bhagwat, Identification of N-(5-tert-butyl-isoxazol-3-yl)-N′-{4-[7-(2-morpholin-4-yl-ethoxy)imidazo-[2,1-b][1,3]benzothiazol-2-yl]phenyl}urea dihydrochloride (AC220), a uniquely potent, selective, and efficacious FMS-like tyrosine kinase-3 (FLT3) inhibitor, J. Med. Chem. 52 (2009) 7808–7816, http://dx.doi.org/10.1021/jm9007533.
[10] European Medicine Agency, Guideline on bioanalytical method validation, 2011. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf (Accessed 10 May 2016).
[11] Centre for Drug Evaluation and research of the U.S. Department of Health and Human Services Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation, 2001. http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm064964.htm (Accessed 10 May 2016).
[12] J.J.M. Rood, M.T.J. van Bussel, J.H.M. Schellens, J.H. Beijnen, R.W. Sparidans, Liquid chromatography–tandem mass spectrometric assay for the T790M mutant EGFR inhibitor osimertinib (AZD9291) in human plasma, J. Chromatogr. B 1031 (2016) 80–85.
[13] S. Kollipara, G. Bende, N. Agarwal, B. Varshney, J. Paliwal, International guidelines for bioanalytical method validation: a comparison and discussion on current scenario, Chromatographia 73 (2011) 201–217.
[14] M.L. Rocci, V. Devanarayan, D.B. Haughey, P. Jardieu, Confirmatory reanalysis of incurred bioanalytical samples, AAPS J. 9 (2007) E336–E343.