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Quantification of FGFR4 inhibitor BLU-554 in mouse plasma and tissue homogenates using liquid chromatography-tandem mass spectrometry

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ABSTRACT

BLU-554 is a potent, highly selective oral FGFR4 inhibitor. A bioanalytical assay for quantification of BLU-554 in mouse plasma and six tissue homogenates (brain, kidney, liver, lung, small intestine, and spleen) was developed and validated using liquid chromatography with tandem mass spectrometric detection and with erlotinib as internal standard. After protein precipitation with acetonitrile in a 96-well format and separation on an XBridge® Peptide BEH C18 column by gradient elution using 0.2% (v/v) ammonium hydroxide (in water) and methanol, analytes were ionized by positive electrospray and monitored in the selected reaction monitoring mode by triple quadrupole mass spectrometry. The assay was validated in a 1–1000 ng/ml concentration range using calibration in mouse plasma. Precisions (intra–day and inter–day) were in the range 2.8–10.1% and accuracies were in between 88.5 and 96.6% for all levels in all matrices. The assay was successfully applied for a pilot pharmacokinetic and tissue distribution study in wild-type mice.

1. Introduction

Hepatocellular carcinoma (HCC) is a lethal cancer for which there are only limited targeted therapies because of its complexity [1,2]. For patients with advanced or metastatic HCC, sorafenib was the first multikinase inhibitor to show a significant advancement in clinical results. Sorafenib, however, only provides minimal survival benefit for patients [3]. As a result of recent advances in genomic analysis, the fibroblast growth factor 19 (FGF19) was identified as a key HCC driver. Signalling pathways involving its receptor, the fibroblast growth factor receptor 4 (FGFR4) was found to be changed in a subset of HCC patients [4–6]. FGF19 is a hormone-like protein and is predominantly expressed by mature hepatocytes. FGF19 is involved in liver homeostasis through its interaction with FGFR4 under normal physiological conditions. The overexpression of FGF19 was found in ca. 20% of HCCs and several compounds directed against FGFR4 are in development [7]. So far, > 20 FGFR4 inhibitors including BLU-554 and FGR401 have been studied in phase I and II clinical trials for HCC treatment [8,9].

BLU-554 (Fig. 1; N-((3*S*, 4*S*)-3-((6-(2, 6-dichloro-3,5-dimethoxyphenyl)quinazolin-2-yl)amino)tetrahydro-2H-pyran-4-yl)acrylamide) is a potent, exquisitely selective, covalently binding inhibitor of FGFR4. BLU-554 is currently evaluated in a phase I clinical trial for the treatment of patients with advanced HCC that have been pre-treated. A phase I study has shown that BLU-554 is well tolerated at the recommended dose of 600 mg once daily [10]. BLU-554 also demonstrated important anti-tumour activity in FGF19 immunohistochemistry (IHC) + advanced HCC patients.

A literature survey suggested that bioanalytical studies for BLU-554 have not been reported hitherto. A bioanalytical assay for this drug will be indispensable for future clinical and preclinical studies. This paper describes the development and validation of an LC-MS/MS method in a high-throughput 96-well format for the quantitative analysis of BLU-

Abbreviations: FGFR, fibroblast growth factor receptor; HCC, hepatocellular carcinoma; IS, internal standard; LC − MS/MS, liquid chromatography-tandem mass spectrometry; LLOQ, lower limit of quantification; QC, quality control; R^2, coefficient of determination; SD, standard deviation; SRM, selected reaction monitoring

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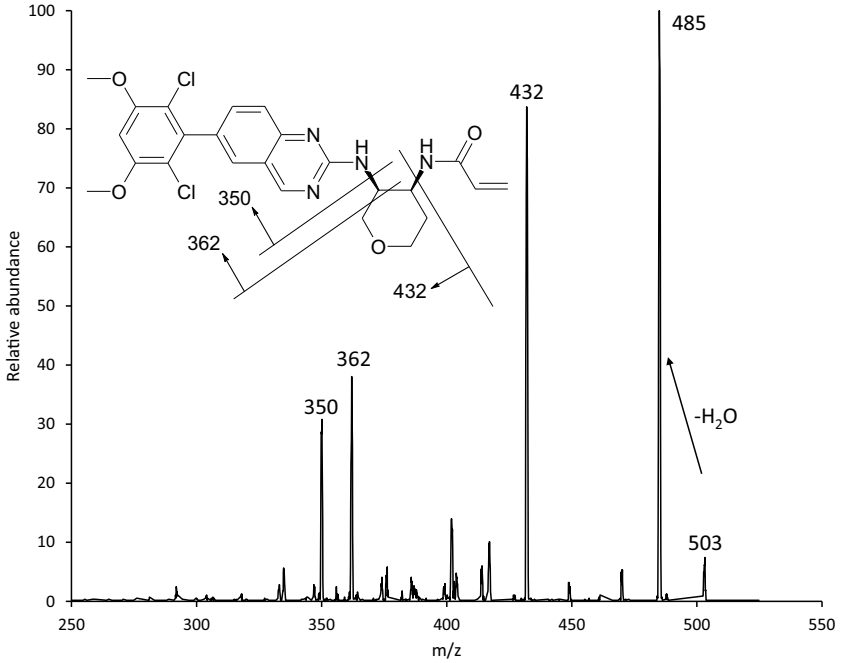

Fig. 1. Chemical structure and product spectrum (m/z 503.1@43 V) of the protonated molecule of BLU-554 ($[M + H]^+$) with the expected fragments.

554 in mouse plasma and homogenates of six different tissues: brain, kidney, liver, lung, small intestine, and spleen, to support preclinical studies with this drug.

2. Material and methods

2.1. Chemicals and reagents

BLU-554 (> 99%) was supplied by Carbosynth (Compton, Berkshire, UK). Erlotinib (> 99%, as hydrochloric acid), used as internal standard (IS), was obtained from the same company under its previous name Sequoia Research Products (Pangbourne, UK). Methanol of HPLC quality, and water of ULC-MS quality (for preparation of eluent) were acquired from Biosolve (Valkenswaard, The Netherlands). For all other purposes, water was purified by reversed osmosis on a multi-laboratory scale. Analytical grade ammonium hydroxide was supplied by Sigma-Aldrich (Steinheim, Germany). Pooled CD-1 mouse lithium heparin plasma (female) and human lithium heparin plasma (mixed gender) were from Sera Laboratories (Haywards Heath, West Sussex, UK).

2.2. Tissue homogenization

The blank mouse tissue homogenates were acquired by stirring ice-cold 4% (w/v) bovine serum albumin in water with whole (weighed) organs. Using the FastPrep-24™ 5G instrument (M.P. Biomedicals, Santa Ana, CA, USA) for 1 min, a homogenized mixture was prepared. The volumes of albumin solution used were 2 ml for two kidneys (ca. 0.4 g), 3 ml for a liver (ca. 1.7 g) and small intestine (ca. 1.0 g), and 1 ml for brain (ca. 0.5 g), two lungs (ca. 0.15 g), and spleen (ca. 0.1 g).

2.3. Analytical instruments

The Shimadzu (Kyoto, Japan) Nexera X2 chromatographic system equipped with a DGU-20A5R degasser, two LC30-AD pumps, a Sil30-ACmp autosampler, and a CTO-20 AC column oven was used. The triple quadrupole mass spectrometer AB SCIEX QTRAP® 5500 (Ontario, Canada), equipped with a Turbo V™ TurboIonSpray® probe and an inlet valve, was used for detection. Analyst 1.6.2. (Sciex) software was used for data collection and control of the LC system and mass spectrometer. MultiQuant 3.0.1 (Sciex) was used for processing all LC-MS/MS data.

2.4. LC-MS/MS conditions

Partial loop injections (1 µl) were applied on an XBridge® Peptide BEH C18 column (50 × 2.1 mm, d_p = 3.5 µm, Waters, Milford, USA) protected by a XBridge® BEH C18 VanGuard cartridge (5 × 2.1 mm, d_p = 3.5 µm, Waters). The column temperature was maintained at 40 °C and autosampler racks at 4 °C. The gradient elution was performed at 0.5 ml/min with (A) 0.2% (v/v) ammonium hydroxide in water and (B) methanol. After injection the percentage of methanol was increased linearly from 45 to 75% in 1.5 min, followed by flushing at 100% methanol for 0.5 min. Finally, the column was reconditioned at the initial conditions for 1.0 min. The eluent was transferred to the electrospray by the divert valve from 1.0 to 2.0 min after injection.

Parameters for selected reaction monitoring (SRM) detection in positive mode were: curtain gas (CUR), 25 psi; collision gas (CAD), medium; ion-spray voltage (IS), 2500 V; temperature (TEM), 700 °C; ion source gas 1 (GS1), 50 psi; ion source gas 2 (IS2), 70 psi; entrance potential (EP), 11 V. Mass transitions were monitored at 150 ms dwell times and unit mass resolutions, their individual parameters are listed in Table 1. Optimized parameters were obtained by direct infusion of 10 ng/ml BLU-554 (and erlotinib separately) at 10 µl/min in methanol/0.2% ammonium hydroxide in water (50/50, v/v) and additionally by direct infusion of 100 ng/ml of an individual compound (10 µl/min)

Table 1
MS/MS parameters for individual SRM transitions of BLU-554 and the internal standard erlotinib.

Compound	m/z Q1	m/z Q3	DP (V)	CE (V)	CXP (V)
BLU-554	503.1	485.1	80	29	22
		432.1[a]	80	43	18
		350.1	80	49	16
Erlotinib	394.1	278.1	50	47	12

DP: declustering potential, CE: collision energy, CXP: collision cell exit potential,

[a] Product used for quantification.

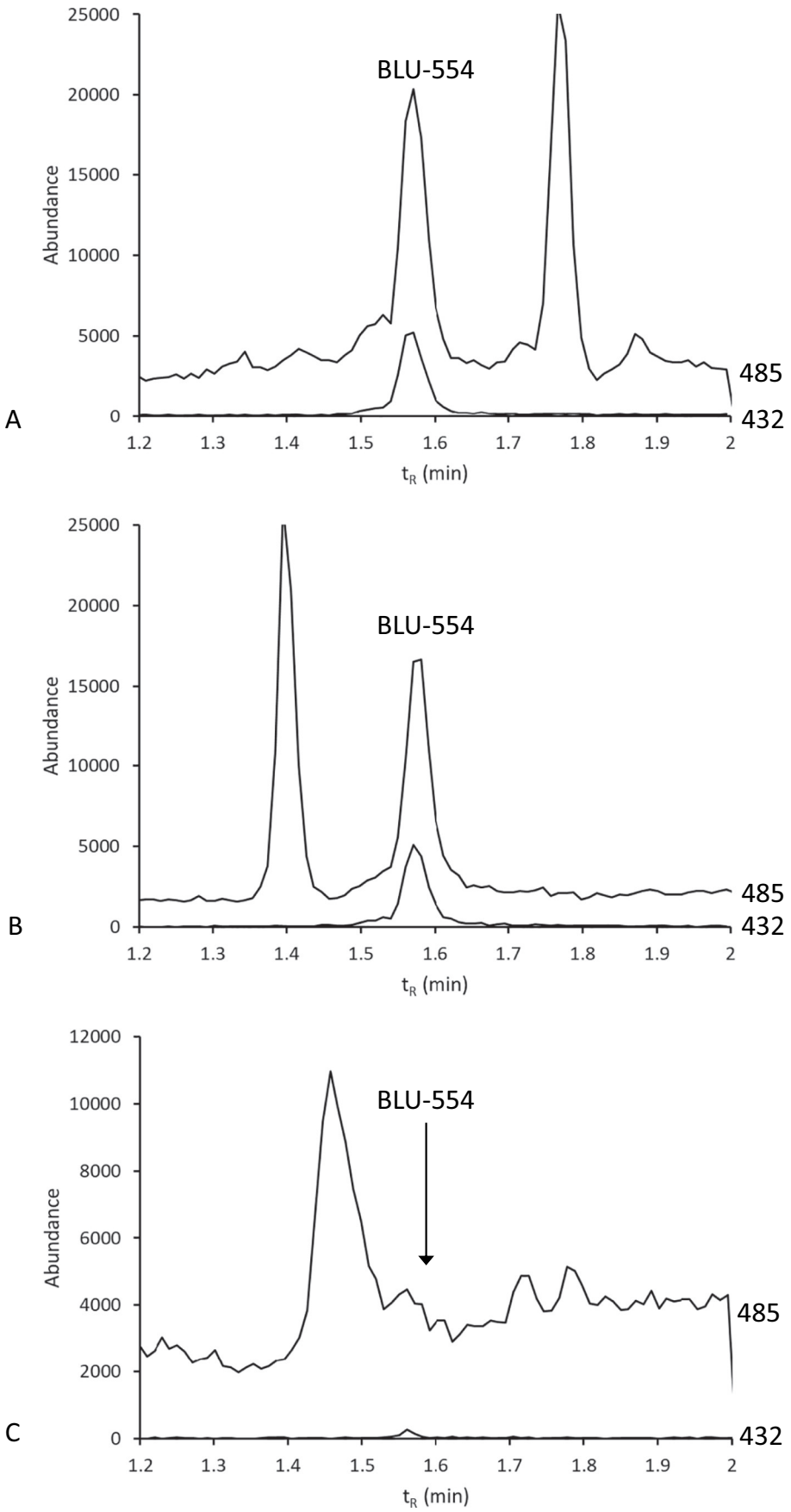

Fig. 2. SRM chromatograms (m/z 503.1 → 485.1 & 432.1@29 & 43 V) for (A) plasma spiked at 2.5 ng/ml BLU-54, (B) small intestine homogenate spiked at 2.5 ng/ml BLU-54 and (C) blank pooled plasma.

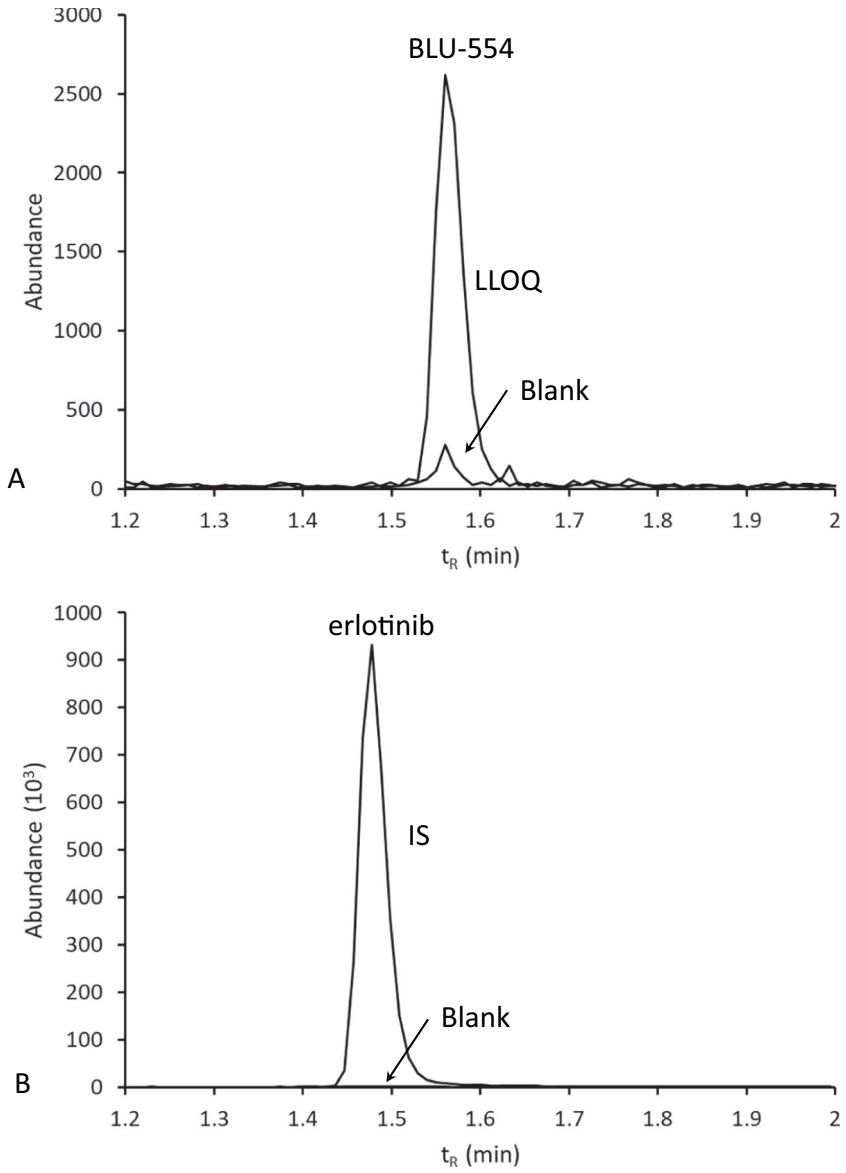

Fig. 3. SRM chromatograms of double blank and LLOQ spiked plasma of (A) BLU-554 (m/z 503.1 → 432.1@43 V) and (B) the IS erlotinib (394.1 → 278.1@47 V) in mouse plasma.

Table 2
Assay performance data (n = 18, 3 days) of BLU-554 in mouse plasma.

Level (ng/ml)	Intra-day precision (%)	Inter-day precision (%)	Accuracy (%)
750	5.5	5.5	91.7
50	3.8	4.1	91.1
2.5	7.2	9.8	96.6
1	8.5	10.1	91.6

Table 3
Assay performance data (n = 18, 3 days) and stability (recovery after 6 h storage at ambient temperature) of 50 ng/ml BLU-554 in tissue homogenates.

Tissue	Intra-day precision (%)	Inter-day precision (%)	Accuracy (%)	Recovery [%]
Brain	2.8	4.6	90.4	100.2 ± 3.0
Kidney	5.6	6.2	96.4	95.12 ± 1.4
Liver	3.7	5.6	88.5	97.2 ± 2.7
Lung	5.5	9.4	91.1	89.0 ± 2.6
Small intestine	5.2	6.7	92.8	97.4 ± 3.6
Spleen	6.1	7.6	90.5	98.8 ± 3.7

mixed with 500 µl/min of the same water-methanol-ammonium hydroxide solvent mixture.

2.5. Stock and working solutions

The stock solutions of BLU-554 were prepared in methanol at concentration of 0.5 and 1 mg/ml. The stock solution of 1 mg/ml erlotinib was prepared in methanol. Working solutions of BLU-554 were obtained by diluting the stock solution 20-fold with water/methanol (1/1; v/v) to 25 and 50 µg/ml. A working solution of erlotinib was obtained by diluting the stock solution 100-fold with water/methanol (1/1; v/v) to 10 µg/ml. All solutions were stored at −30 °C. The erlotinib extraction solution was prepared by diluting 250-fold to 40 ng/ml in acetonitrile.

2.6. Calibration standards and quality control samples

The highest calibration sample was prepared by diluting the 50 µg/ml BLU-554 working solution to 1000 ng/ml in blank mouse plasma in

Table 4
Stability data (recovery [%]; ± S.D.; $n = 4$) of BLU-554 in mouse plasma, reporting the percentage of the initial concentration.

Condition	QC-high	QC-low
3 freeze-thaw cycles	101.4 ± 5.0	95.8 ± 5.3
24 h at ambient temperature	97.06 ± 5.45	86.70 ± 9.12
3.5 months at −30 °C	94.1 ± 0.8	98.8 ± 4.5

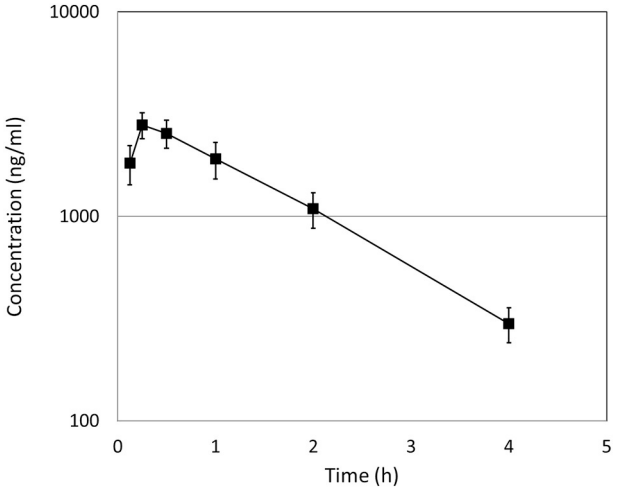

Fig. 4. Pharmacokinetic curve of BLU-554 in FVB/NRj mice ($n = 6$) after oral administration of 10 mg/kg.

polypropylene tubes. Until further use, this sample was stored at −30 °C. This calibration sample was diluted further for daily calibration with blank human plasma to 500, 100, 50, 10, 5, 2, and 1 ng/ml in mouse plasma. Quality control (QC) samples were prepared at 750 (high), 50 (medium), 2.5 (low) and 1 ng/ml (lower limit of quantitation (LLOQ)) in mouse plasma from the 25 µg/ml working solution by sequential dilution and at 3750 ng/ml by direct dilution of the 0.5 mg/ml stock solution with mouse plasma. QC samples at 50 ng/ml (medium) were also prepared in pooled mouse tissue homogenates of brain, liver, lung, small intestine, and spleen and in FVB/NRj kidney homogenate.

2.7. Sample preparation

Ten µl of plasma or tissue homogenate was pipetted into a polypropylene 96-well plate with a 200 µl conical bottom and 20 µl of 40 ng/ml erlotinib in acetonitrile was added. The 96-well plates were closed with a silicone mat and shaken by vortex mixing. Next, the plate was centrifuged for 5 min at 2643 × *g* and 20 µl of the supernatant was transferred to a 96-deep well plate with a 1-ml round bottom. After adding 200 µl of 25% (v/v) methanol in water, samples were mixed by manual shaking gently. The prepared plate was located in the autosampler rack for injection of 1 µl of the final sample.

2.8. Bioanalytical method validation

In the 1–1000 ng/ml range the quantification was validated using international guidelines [11,12] with full validation for mouse plasma, and partial validation for the six tissue homogenates.

2.8.1. Calibration

All calibration samples were prepared in duplicate for each calibration together with additional blank (no analyte) and double blank (no analyte and no erlotinib) control samples. Weighted quadratic least-squares regression with $1/x^2$ (x is the concentration of BLU554) as the weighting factor was used for calculations from the peak area of BLU-554 relative to the peak area of erlotinib.

2.8.2. Precision and accuracy

The QC-high (750 ng/ml), −medium (50 ng/ml), −low (2.5 ng/ml), and -LLOQ (1 ng/ml) samples prepared in mouse plasma and six tissue homogenates were used to assess the precision and accuracy of the assay. Precisions (inter− and intra−day) and accuracies were determined in three separate runs of 6 replicates each for all samples. Relative standard deviations were calculated for the within- and between-day precisions. Dilution integrity was tested for high drug levels by diluting 10 µl of the 3750 ng/ml plasma QC sample (6 replicates, inter-day) with 40 µl of blank human plasma (1:5 dilution) before further treatment of 10 µl of the diluted mixture.

2.8.3. Selectivity

The selectivity of the assay was assessed for eight individual mouse plasma and 16 tissue homogenates (2 brain and spleen, 3 liver, kidney, lung, and small intestine). Each sample was analysed as double blank (no analytes, no IS), and as LLOQ spiked sample (1 ng/ml BLU-554).

2.8.4. Recovery and matrix effect

Recovery and matrix effect of BLU-554 in mouse plasma were assessed by preparing three types of samples at three levels with four replicates each. Normal plasma treatment was performed (A) and in addition similar samples with the drug added after extraction (B) and samples without any matrix constituents at the same levels (C) were analysed. Recovery was calculated from ratio A/B and matrix effect from C/B. Similar samples and calculations were used for the IS.

Relative matrix effects were studied for the same samples used for selectivity: 8 plasma and 16 tissue homogenate samples. The blank extracts were supplemented with the drug at the high and low level, respectively, mixed with the IS. The relative matrix effect was calculated by comparison with a matrix free reference solutions containing both compounds at the same concentrations.

2.8.5. Stability

The stability of BLU-554 was investigated in mouse plasma at QC-high and -low levels (all $n = 4$). The separate portions of 10 µl in 200-µl well plates were kept under different conditions. Tested conditions were ambient temperature (ca. 22 °C) for 24 h, three additional freeze-thaw cycles at ambient temperature (thawing at ambient temperature for 1.5 h, and freezing again at least for one day at −30 °C), and − 30 °C for 3.5 months. Stability in pooled tissue homogenates (brain, liver, lung, small intestine, spleen) and FVB/NRj kidney homogenate was investigated at the medium level at ambient temperature for 6 h.

Drug stability in stock solutions (in methanol) and working solutions (in 50% (v/v) methanol) at ambient temperature for 6 h and at −30 °C for 3 months was tested (all $n = 2$ with duplicate preparation and duplicate injection) by comparison with fresh solutions after proper dilution and adding IS using LC-MS/MS.

A complete validation run with diluted extracts of calibration and 24 QC samples was analysed after 10 day storage at 4 °C to test autosampler stability.

2.9. Pharmacokinetics and tissue distribution of BLU-554 in mice

2.9.1. Mouse treatment

A pharmacokinetic pilot study was carried out in wild type male mice (FVB/NRj genetic background) administering 10 mg/kg BLU-554 orally ($n = 6$). BLU-554 was dissolved in dimethyl sulfoxide (50 mg/ml), followed by 2.5-fold dilution with polysorbate 80/ethanol (1:1, v/v), and then 20-fold dilution with a 10 mM hydrochloric acid solution to obtain a drug working solution of 1 mg/ml, prepared freshly on each day of an experiment. Mice were housed and handled according to institutional guidelines complying with Dutch legislation and treated

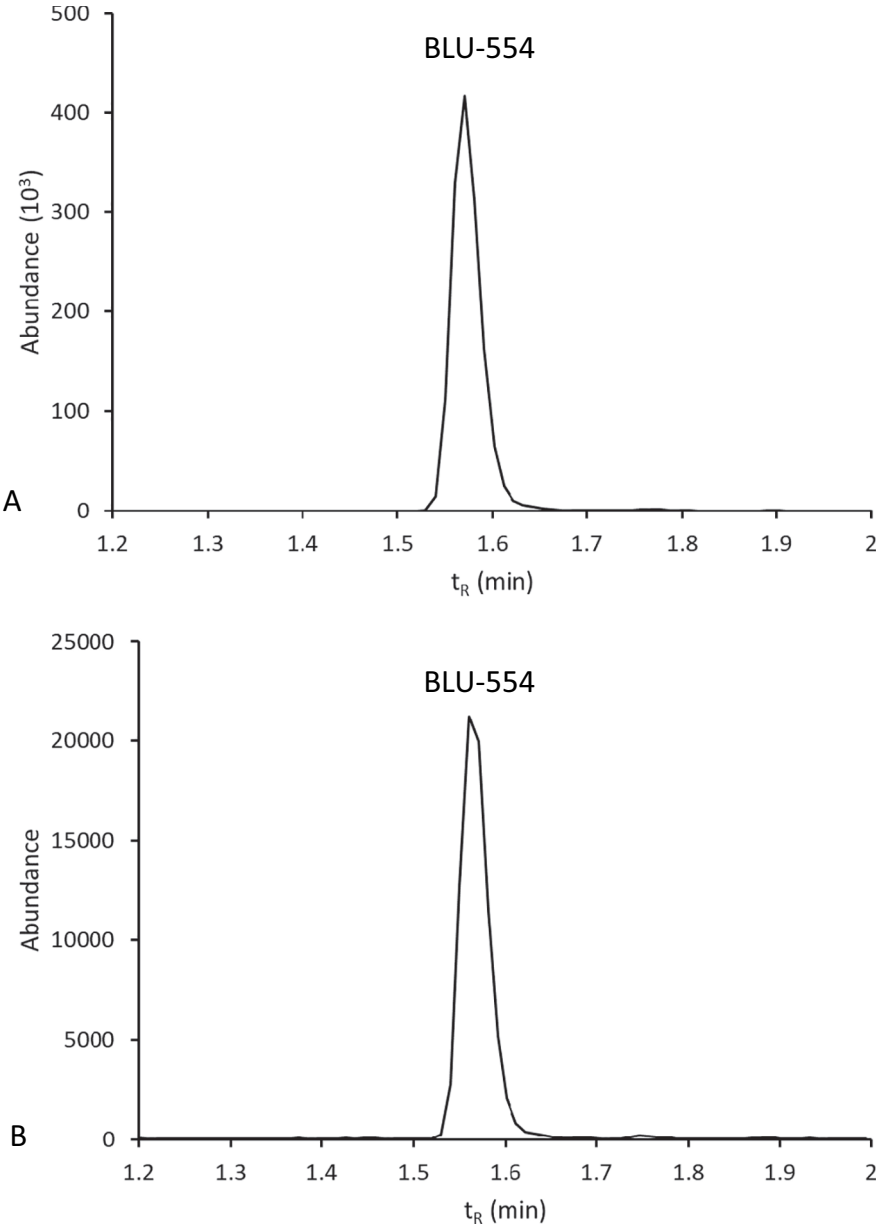

Fig. 5. SRM chromatograms (m/z 503.1 → 432.1@43 V) of BLU-554 in (A) plasma (230 ng/ml) and (B) brain homogenate (9.5 ng/ml), 4 h after administration of 10 mg/kg to a FVB/NRj mouse.

similar to earlier reported protocols [13]. Shortly, mice were 11 to 14 weeks of age and housed in a temperature-controlled environment with a 12-h light/12-h dark cycle. Animals were administered a standard diet and acidified water and were fasted for 2–3 h before BLU-554 was administered by gavage into the stomach, using a blunt-ended needle. At 7.5, 15, 30 min, 1, and 2 h after administration, blood was collected from the tail vein in heparinized capillary tubes (Sarstedt, Germany). After 4 h, isoflurane used as anaesthetic agent to narcotize the mice and a final blood sample was obtained with cardiac puncture. Plasma was acquired by centrifugation at 9000 ×*g* for 6 min at 4 °C and stored at −30 °C until analysis. The mouse plasma samples from 0.125 to 2 h were diluted 5 fold with human lithium heparin plasma before analysis. The further analytical process has been reported above.

2.9.2. Pharmacokinetic calculations

Pharmacokinetic parameters were calculated as mean ± standard deviation (SD). The time to reach the maximum concentration (T_{max}) and maximum plasma levels (C_{max}) were both calculated directly from the highest concentrations and their time points, the elimination half-life ($T_{1/2}$) was calculated from $C_{0.5}$ to C_4. The area under the plasma concentration-time curve during the 4-h experiment ($AUC_{0\rightarrow4}$) and the extrapolated value ($AUC_{0\rightarrow\infty}$) were calculated using the trapezoidal rule and extrapolation from 4 h to infinity using the elimination rate and the concentration at 4 h. Finally, apparent clearance (Cl/F) and apparent volume of distribution (V_d/F) were both calculated by using the first-order one-compartment model.

2.9.3. Incurred samples reanalysis

All 4-h samples (n = 6 for each of 6 matrices) were reanalysed within a few days.

3. Results and discussion

3.1. Method development

In chromatographic bioanalysis with LC-MS/MS protein

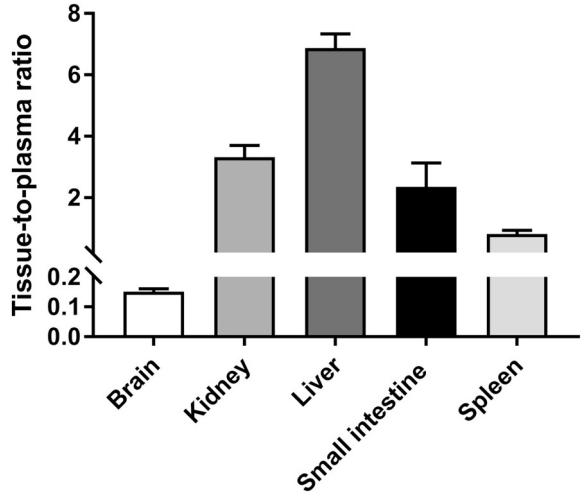

Fig. 6. Tissue − to − plasma concentration ratios (ng/g to ng/ml) in FVB/NRj mice (n = 6) in 5 tissues, 4 h after oral administration of 10 mg/kg of BLU-554.

precipitation with acetonitrile is a widely used and efficient sample pretreatment method for kinase inhibitors [14] that was also applicable for BLU-554. This method is simpler than some extraction methods including solid-phase and liquid-liquid extraction. Further, the application of protein precipitation in a 96-well format is a convenient approach to enhance sample throughput. With the present approach hundreds of samples can be prepared in a few hours and analysed within 24 h. The positive electrospray settings were optimized for the single protonated BLU-554 (m/z 503.1) to obtain a maximal response. A product spectrum of BLU-554 is shown in Fig. 1 with the proposed fragmentation products. The product m/z 432.1 was chosen for quantification because the main product formed by water loss (m/z 485.1) shows a high noise in the background signal and, depending on the matrix, additional peaks that may interfere with the quantification of the analyte (Fig. 2).

The chromatographic method was developed on the XBridge® Peptide BEH C18 column based on MS response, retention time and peak shape. An alkaline resistant column was preferred because under acidic conditions (formic acid) peak broadening was relatively high and response was lower. Further, acetonitrile as organic modifier also resulted in a lower response for the analyte compared to methanol, so the combination of methanol and ammonium hydroxide showed maximal electrospray ionization efficiency for BLU-554. The amount of ammonium hydroxide did not show any significant influence on the MS response and chromatographic performance.

Erlotinib was selected as IS after screening of different compounds with similar chromatographic properties as BLU-554. A product spectrum of erlotinib was reported previously by us [15]. A stable isotopically labelled analogue of BLU-554 was not available for our studies.

3.2. Validation

Based on the LC-MS/MS response, the calibration range was chosen from 1 to 1000 ng/ml. Because levels exceeding this range may also be expected, dilution integrity was included in the validation program. Representative chromatograms of BLU-554 and erlotinib in mouse plasma are shown in Fig. 3.

3.2.1. Calibration

The relative responses of BLU-554 showed non-linearity at higher concentrations, therefore, least-squares quadratic (second degree polynomial) regression was used as an alternative for linear regression. The calibration results were given as quadratic model ($y = A + B{\cdot}x + C{\cdot}x^2$) with parameters A (intercept), B (slope) and C (quadratic factor), the coefficient of determination (R^2) and y being target peak area relative to IS and x the BLU-554 concentration in ng/ml. Calibration parameters are reported as mean ± SD ($n = 5$). The average equation was obtained was $y = 6.45\ (\pm 3.65){\cdot}10^{-4} + 2.37\ (\pm 0.13){\cdot}10^{-3}x - 9.16\ (\pm 5.26){\cdot}10^{-8}x^2$ with $R^2 = 0.997(\pm 0.003)$.

3.2.2. Precision and accuracy

Assay performance data of the QC samples are reported at four levels for mouse plasma samples (Table 2) and the medium level for each of the tissue homogenates (Table 3). Intra-day precision, inter-day precision, and accuracy at all levels and matrices were within ± 15% for QC − high, − medium and − low and ± 20% for QC-LLOQ as required by the guidelines [11,12]. Dilution integrity was obtained with a 3.3% precision and a 97.2% accuracy at 3750 ng/ml BLU-554 with 5 fold-dilution in human plasma.

3.2.3. Selectivity

None of the blank BLU-554 responses (8 plasma samples; 16 tissue homogenates) exceeded 20% of the LLOQ (0.2 ng/ml BLU-554) allowed by current guidelines [11,12]. Blank erlotinib responses did not exceed 0.13% of the normal IS response. Levels measured in LLOQ spiked samples were 0.902 ± 0.035 ng/ml in plasma ($n = 8$) and 0.984 ± 0.100 ng/ml in tissue homogenates ($n = 16$) with only one kidney homogenate sample exceeding 120% of the target value.

3.2.4. Recovery and matrix effect

Data (all $n = 4$) for recovery (103.0 ± 5.0%, 102.3 ± 3.4%, 101.1 ± 2.3%, 103.4 ± 1.1%) and matrix effect (103.2 ± 0.8%, 103.9 ± 1.4%, 103.0 ± 4.2, 102.8 ± 1.9%) in plasma at high, medium and low BLU-554 QC levels and for IS were excellent. No significant extraction losses or matrix effects were observed. The relative matrix effect in individual blank samples ($n = 24$, 8 plasma samples and 16 tissue homogenates) showed similar results (100.5 ± 1.4% and 98.7 ± 3.6% at high and low QC levels).

3.2.5. Stability

Results of BLU-554 stability in mouse plasma are shown in Table 4, while tissue homogenate results are shown in Table 3, no degradation was observed under all conditions. Stock and working solutions also were stable; recoveries for stock solutions were 98.4% after 3 months at − 30 °C and 100.2% after 6 h at ambient temperature, recoveries under the same conditions were 100.6% and 99.2% for working solutions. Finally, measuring a compete analytical run after 10 days at 4 °C resulted in a successful performance without any QC sample ($n = 24$) exceeding ± 15%.

3.3. BLU-554 in mice

3.3.1. Pharmacokinetics

A pharmacokinetic curve for BLU-554 in plasma from 6 wild-type (FVB/NRj) mice is shown in Fig. 4, a representative chromatogram is shown in Fig. 5A. Within-subject variations were small and absorption was fast, the following parameters could be calculated: T_{max} = 0.29 ± 0.10 h, C_{max} = 2833 ± 408 ng/ml, $T_{1/2}$ = 1.14 ± 0.14 h, $AUC_{0\rightarrow 4}$ = 5072 ± 823 ng·h·ml^{-1}, $AUC_{0\rightarrow\infty}$ = 5571 ± 851 ng·h·ml^{-1}, Cl/F = 1832 ± 296 ml·h^{-1}·kg^{-1}, and V_d/F = 3042 ± 783 ml/kg.

3.3.2. Tissue distribution

Tissue distribution of BLU-554 in wild-type mice is shown in Fig. 6 and a chromatogram of BLU-554 in brain homogenate is shown in Fig. 5B. Tissue concentrations decreased in the order liver > kidney > small intestine > spleen > brain. Concentrations were much lower in brain, most likely because of the presence of the blood-brain barrier. This barrier shows high expression of some ABC efflux transporters, such as p-glycoprotein (P-gp; MDR1; ABCB1) and breast

cancer resistance protein (BCRP; ABCG2). The high BLU-554 liver-to-plasma ratio suggests there is a relatively high amount of the drug being transported into the liver. Since BLU-554 is primarily applied for HCC, this might be a beneficial feature of this drug.

3.3.3. Incurred samples reanalysis

Reanalysis of all 4 h samples of the pilot study in wild-type mice (n = 36) resulted for only one plasma sample in a difference exceeding 20%. A success rate exceeding the 33% of the guidelines [11,12] by far.

4. Conclusions

The first bioanalytical assay for selective oral FGFR4 inhibitor BLU-554 has been developed and reported in a successful validation using only 10 µl sample volume. The assay includes a simple, efficient and rapid sample pre-treatment approach which comprises protein precipitation with acetonitrile in a 96-well format. The precision, accuracy at all levels and matrices were found as required in the international guidelines. The results showed no significant extraction losses or matrix effects. The new LC-MS/MS method was successfully used for a pharmacokinetic and tissue distribution study in mice and could be further applied in mouse studies.

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