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A validated assay for the quantitative analysis of vatalanib in human EDTA plasma by liquid chromatography coupled with electrospray ionization tandem mass spectrometry

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ABSTRACT

A sensitive and accurate method for the determination of vatalanib in human EDTA plasma was developed using high-performance liquid chromatography and detection with tandem mass spectrometry. Stable isotopically labeled imatinib was used as internal standard. Plasma proteins were precipitated and an aliquot of the supernatant was directly injected onto a Phenomenex Gemini C18 analytical column (50 mm × 2.0 mm ID, 5.0 μm particle size) and then compounds were eluted with a linear gradient. The outlet of the column was connected to a Sciex API 365 triple quadrupole mass spectrometer and ions were detected in positive multiple reaction monitoring mode. The lower limit of quantification was 10 ng/mL (S/N ≈ 10, CV ≤ 8.4%). This method was validated over a linear range from 10 to 2500 ng/mL, and results from the validation study demonstrated a good intra- and inter-assay accuracy (inaccuracy ≤9.57%) and precision (CV ≤ 8.81%). This method has been used to determine plasma vatalanib concentrations in patients with advanced solid tumor, enrolled in a phase I pharmacokinetic trial with the drug.

1. Introduction

Neovascularization of tumor tissue is essential for tumor growth and metastasis formation. The vascular endothelial growth factor (VEGF) is a multifunctional cytokine involved in neovascularization by increasing vascular permeability and stimulating endothelial cell growth and angiogenesis. VEGF is secreted by tumor cells and macrophages and evokes its effects by binding to cell surface VEGF receptors (VEGFR) on the tumor vascular endothelium [1–3]. The family of VEGFRs consists of three different tyrosine kinase receptors (VEGFR-1, VEGFR-2 and VEGFR-3). Specific inhibition of angiogenesis by blocking tyrosine kinase receptors could prevent the growth of tumors and their metastatic potential. Since cell division of endothelial cells in the normal vasculature is rare, inhibition of angiogenic signals by interfering with VEGFR-induced signals selectively targets the vasculature of tumor tissue. Therefore VEGFR targeted therapy is expected to be well tolerated in cancer patients [1,3].

Vatalanib (PTK787/ZK222584) belongs to the class of aminophtalazines and is a potent orally active tyrosine kinase receptor inhibitor blocking all known VEGFRs, with a greater potency against VEGFR-1 and VEGFR-2. In addition, vatalanib also inhibits other tyrosine kinase receptors, such as the platelet-derived growth factor receptor (PDGF), c-kit and c-Fms [3,4]. The intended indication for use of vatalanib is tumors with an overexpression of VEGF or VEGFR. Currently, vatalanib is studied in phase I, II and III trials in several advanced solid tumors, including cancer of the gastrointestinal tract, prostate, breast, ovary, lung, liver and brain [4].

To support the pharmacokinetic analysis in clinical trials, compound specific analytical methods are essential. Analytical methods based on high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS) have become irreplaceable in the quantitative analysis of small molecules in biological matrices [5]. However, to date only HPLC–UV methods for determination of vatalanib have been reported in literature [6,7]. Therefore we developed and validated a sensitive and specific HPLC–MS/MS method for quantification of vatalanib in human EDTA plasma.

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2. Experimental

2.1. Chemicals and materials

Reference standards and internal standards were provided by the following manufacturers: vatalanib·HCl ($C_{20}H_{15}ClN_4$·HCl) by Sequoia Research Products (Oxford, United Kingdom), imatinib-$^{13}C,^2H_3$·($C_{29}H_{31}N_7O$) stable isotope by Alsa Chim (Illkirch, France). HPLC-grade acetonitrile and methanol were purchased from Biosolve (Valkenswaard, The Netherlands). Distilled water was obtained from B.Braun (Melsungen, Germany). Ammonia 25% was purchased from Merck (Darmstadt, Germany). Blanco plasma with EDTA as anticoagulant was obtained from Slotervaart Hospital (Amsterdam, The Netherlands).

2.2. Mass spectrometric conditions

An API 365 triple quadrupole mass spectrometer (Sciex, ON, Canada) equipped with an electrospray ionization (ESI) source (Sciex) operating in the positive ion mode was used as detector. The ionization source parameters were: nebulizer gas, 10 arbitrary units (a.u.); curtain gas, 6 a.u.; ionspray voltage, 5000 V; heater gas, 350 °C; turbo gas, 7 L/min. The nebulizer (1.8 L/min) and turbo (7.0 L/min) gases were zero air, while curtain (1.3 mL/min) and collision activated dissociation gas (240×10^{12} molecules/cm^2) consisted of nitrogen (grade 5.0). The dwell time was 150 ms with a 5-ms pause between scans. Quadruples operated at unit mass resolution (0.7 Da). Multiple reaction monitoring (MRM) chromatograms were used for quantification. Mass-transitions of from m/z 347 to 311 for vatalanib and m/z 498 to 394 for the internal standard imatinib-$^{13}C,^2H_3$ were optimized. Data were processed by Analyst software (version 1.4, Sciex).

2.3. Chromatographic conditions

Chromatographic separations of vatalanib and the internal standard were carried out using an Agilent 1100 HPLC system (Agilent technologies, Palo Alto, CA, USA) consisting of a binary pump and a cooled autosampler (4 °C). Compounds were eluted on a linear gradient at a flow rate of 250 μL/min. Eluent A consisted of 10 mM ammonium hydroxide in water and eluent B of 1 mM ammonium hydroxide in methanol. At time zero, 55% of eluent B was flushed through the column. After 0.5 min, 80% of eluent B was mixed with 20% of eluent A and this mobile phase composition was maintained for 3 min. Then the gradient composition was changed to a mix of 95% eluent B with 5% of eluent A. This composition was maintained for 1 min and the column was reconditioned for 3.4 min with 55% of eluent B before the next injection. Separation was carried out on a reversed phase system with a Gemini C_{18} column (50 mm × 2.0 mm ID, 5.0 μm particle size; Phenomenex, Torrance, CA, USA) protected with a Securityguard Gemini precolumn (4 mm × 2.0 mm ID, 5.0 mm particle size; Torrance, CA, USA) and thermostatted at 40 °C. The column outlet was connected to the ESI through a divert valve. The divert valve was directed to waste during the first 2 min to prevent the introduction of endogenous compounds into the mass spectrometer. The total run time was 8 min and sample injections of 5 μL were carried out.

2.4. Preparation of calibration standards and validation samples

A set of stock solutions of vatalanib was prepared from two independent weightings; one for the calibration standards and one for the validation samples. Approximately 1.1 mg of vatalanib·HCl was accurately weighted and dissolved in 2 mL of methanol in a volumetric flask to give a 500 μg/mL stock solution of the free base. Stock solutions of the internal standard imatinib-$^{13}C,^2H_3$ were made in methanol at a concentration of approximately 1000 μg/mL. A 200 ng/mL working solution of the internal standard was prepared by dilution of the stock solution in methanol. To precipitate the plasma proteins, a mixture of methanol–acetonitrile (1:1, v/v) was used.

For the preparation of the calibration standards, working solutions in the range from 200 to 50,000 ng/mL were used. These working solutions were prepared by dilution of a vatalanib stock solution in methanol. A volume of 50 μL of each working solution was added to 950 μL of control human EDTA plasma to obtain calibration standards in the range from 10 to 2500 ng/mL.

Four working solutions in the range from 200 to 40,000 ng/mL were prepared by dilution of an independently prepared vatalanib stock solution in methanol. To obtain validation samples of 10, 20, 200 and 2000 ng/mL, 50 μL of each working solution was added to 950 μL of control human EDTA plasma. The stock and working solutions in methanol and precipitation reagent were stored at nominally −20 °C until use.

To establish the accuracy and precision of the method when samples are quantified above the upper limit of quantification (ULQ), a sample containing 8000 ng/mL vatalanib was spiked. Before processing, this sample was then diluted 10 times in control human EDTA plasma.

2.5. Sample pre-treatment

Protein precipitation was used as sample pre-treatment for plasma samples with vatalanib and imatinib-$^{13}C,^2H_3$. To 50 μL of plasma sample, 20 μL of internal standard working solution (200 ng/mL) and 150 μL of protein precipitation reagent (−20 °C) were added. After vortex mixing for 15 s, samples were centrifuged at 15,000 × g for 15 min. A volume of 5 μL of the clear supernatant was injected onto the column.

2.6. Validation procedures

A full validation of the assay was performed according to the FDA guidelines for validation of bioanalytical assays including linearity, inaccuracy, precision, specificity, selectivity, cross-analyte/internal standard interference, recovery, ion suppression, carry-over and stability [8,9].

Eight non-zero plasma calibration samples were prepared and analyzed in duplicate in three separate analytical runs. The linear regression of the ratio of the areas of the analyte and the internal standard peaks versus the concentration were weighted. Weighing factors of $1/x$ and $1/x^2$ (where x = concentration) were tested. In order to establish the best weighting factor, back-calculated calibration concentrations were determined. The model with the lowest total bias and the most constant bias across the concentration range was used for further analysis and quantification. The linearity was evaluated by means of back-calculated concentrations of the calibration standards. The deviation from the nominal concentrations should be within ±20% for the lower limit of quantification (LLQ) and within ±15% for the other concentrations with coefficient of variation (CV) values less than 20% and 15% for both the LLQ and the other concentrations, respectively.

Inaccuracy and precision of the assay were established by analyzing validation samples with analyte concentrations at the lower limit of quantification (LLQ) and in the low, mid and high concentration ranges of the calibration curves. Five determinations of each validation sample were measured in three separate analytical runs. Samples with vatalanib concentrations above the ULQ were diluted 1:10 in control human EDTA. These samples were processed in 5-fold and measured in one analytical run to assess the accuracy and precision. The intra-assay inaccuracy was defined as the percentage difference between the mean calculated concentration after three

analytical runs and the nominal concentration. The coefficient of variation (CV%) was used to report the intra- and inter-assay precision. The intra- and inter-assay inaccuracies should be within ±20% for the LLQ and ±15% for all other concentrations. The precisions CV% should be less than 20% for the LLQ and less than 15% for all other concentrations [9].

To investigate whether endogenous compounds from plasma could interfere with the detection of the analyte or the internal standard, six different batches of control drug-free human EDTA plasma prepared as double blanks (containing neither analyte nor internal standard) and LLQ samples. Samples were processed and analyzed according to the described procedures. Areas of peaks co-eluting with the analytes should not exceed 20% of the area at the LLQ level. The deviation of the nominal concentration for the LLQ samples should be within ±20%.

To investigate possible cross interference between vatalanib and the internal standard, a cross interference check was performed. Drug-free human EDTA plasma was spiked at ULQ level and was processed without internal standard. Also drug-free plasma with only internal standard imatinib-$^{13}C,^{2}H_3$ was processed. The response of any interfering peak with the same retention time as vatalanib should be less than 20% of the response of a LLQ sample. The response of any interfering peak with the same retention time as the internal standard imatinib-$^{13}C,^{2}H_3$ should be less than 5% of the response of the internal standard.

The protein precipitation recovery of vatalanib was determined at three concentrations (20, 200 and 2000 ng/mL) by comparing the analytical response of processed samples with those of processed blanks spiked with analyte (representing 100% recovery). The total recovery was determined by comparing the analytical response of processed samples with the analytical response of the unprocessed samples containing only analyte and internal standard in precipitation reagent. Ion suppression (matrix effect) was examined by comparing the analytical response of processed blanks spiked with analyte with those unprocessed samples in precipitation reagent. These experiments were performed in triplicate.

Carry-over was tested by injecting two processed blank matrix samples sequentially after injecting an ULQ sample. The response in the first blank matrix at the retention times of vatalanib and imatinib-$^{13}C,^{2}H_3$ should be less than 20% of the response of a LLQ sample.

The stability of vatalanib in spiked human EDTA plasma after three freeze/thaw cycles from nominally −20 °C to ambient temperatures was investigated in triplicate at two concentrations (20 and 2000 ng/mL) by comparing validation samples that had been frozen and thawed three times with validation samples that had been prepared freshly. The stability of vatalanib in spiked human EDTA plasma maintained at ambient temperatures for 6 h was evaluated in triplicate at two concentrations and compared to validation samples that were kept at −20 °C until processing. The processed sample stability of vatalanib was investigated at two concentrations (20 and 2000 ng/mL). Hereto, the measured concentrations in a final extract of validation samples after 2 days (ambient temperature) and 7 days (2–8 °C) were determined using freshly prepared calibration standards. The re-injection reproducibility was determined after 2 days of storage in the autosampler (4 °C) and compared with the initial concentrations. The analytes were considered to be stable in the matrix or final extract if 85–115% of the initial concentrations was recovered. Stability of stock solutions of vatalanib and imatinib-$^{13}C,^{2}H_3$ stored at ambient temperature for 6 h and at −20 °C was assessed in triplicate. The analyte was considered to be stable in stock solutions if 95–105% of the initial concentration was recovered. The internal standard was considered to be stable if 80–120% of the initial concentration was recovered. Determination of long-term stability of vatalanib in stock solutions and in plasma at −70 °C are currently ongoing.

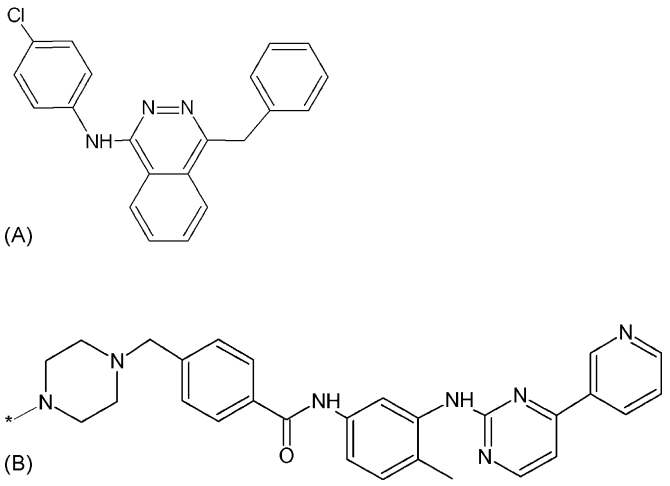

Fig. 1. Chemical structures of vatalanib (A) and imatinib-$^{13}C,^{2}H_3$ (* represents the $^{13}C,^{2}H_3$-group, (B)).

3. Results and discussion

3.1. Mass spectrometry

During optimization of the mass spectrometric parameters, the Q1 spectrum of vatalanib showed the singly charged molecular ion as the most intense ion at *m/z* 347. For imatinib-$^{13}C,^{2}H_3$ the most intense peak in the Q1 spectrum also corresponded to the singly charged molecular ion at *m/z* 498. The structural formulas of vatalanib and imatinib-$^{13}C,^{2}H_3$ are depicted in Fig. 1. MS/MS experiments were carried out to determine the most abundant product ions for multiple reaction monitoring (MRM). MS/MS product ion scans and the proposed fragmentation pathways for the chosen transitions of vatalanib and imatinib-$^{13}C,^{2}H_3$ are shown in Fig. 2. Vatalanib and the internal standard could be detected with the electrospray source operating in the positive mode. Non-linearity was observed for all calibration curves when 2.5 ng of vatalanib or more was injected onto the column. When injecting a smaller volume of 5 μL supernatant the ULQ was set to 2500 ng/mL (injection of 2.8 ng onto the column).

3.2. Chromatography

Due to the basic properties of vatalanib the best retention was observed using an alkaline mobile phase. In order to retain vatalanib on the column and to establish stable retention times a mobile phase with 10 mM ammonium hydroxide (pH 10.2) was applied. Column stability under alkaline conditions was established by successive analyses of more than 500 analytical samples of a pharmacokinetic study. Additional, the number of plates of the column in the first analytical runs did not differ from the number of plates after more than 3 months of extensive column usage.

Since the pH of the aqueous component was ~10, full protonation of the analyte in the mobile phase was not expected. However, the most abundant peaks in the spectrum of vatalanib and imatinib-$^{13}C,^{2}H_3$ were the positively charged molecular ions at *m/z* 347 and 498, respectively. Protonation of compounds during ESI–MS performed with a basic eluent has been described [10].

Peak shape of compounds eluting on a linear gradient starting at 55% eluent B increasing to 100% eluent B in 2.5 min at a flow rate of 250 μL per min was not symmetrical (As ≈ 3). Improved peak shapes (As ≈ 1.1) were obtained when the linear gradient starting on 55% eluent B was followed by an isocratic elution with 80% eluent B at a flow rate of 250 μL per min. To elute the hydrophobic compound from the column and to decrease the carry-over in the HPLC

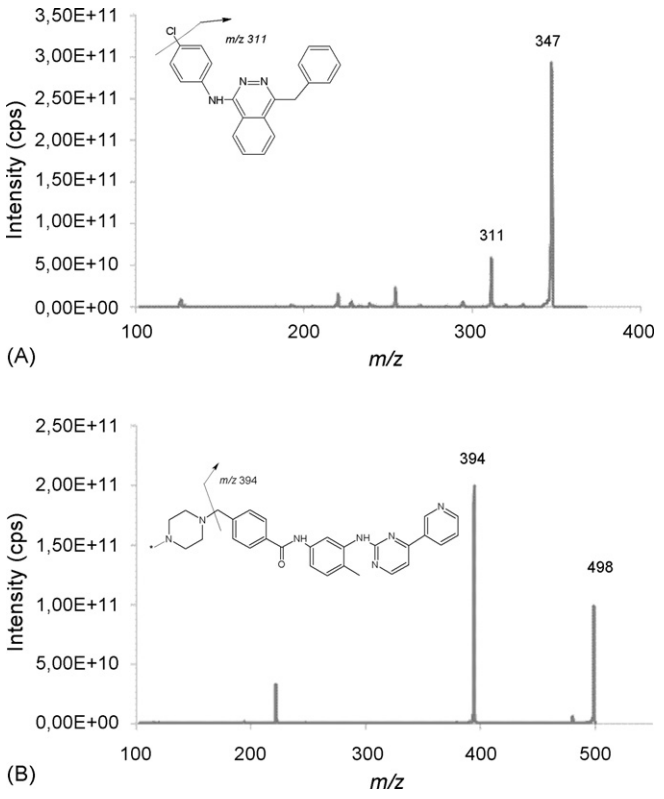

Fig. 2. (A) MS/MS product ion scan of vatalanib (precursor ion *m/z* 347). (B) MS/MS product ion scan of imatinib-$^{13}C,^{2}H_3$ (precursor ion *m/z* 498; * represents the $^{13}C,^{2}H_3$-group).

system the amount of modifier was increased by a quick stepwise gradient to 95% of eluent B after elution of vatalanib. Subsequently, the column was reconditioned with 55% B before the next injection (Fig. 3). Typical chromatograms are depicted in Fig. 4. At LLQ level (10.2 ng/mL) a signal to noise ratio (S/N-ratio) of ±10 was

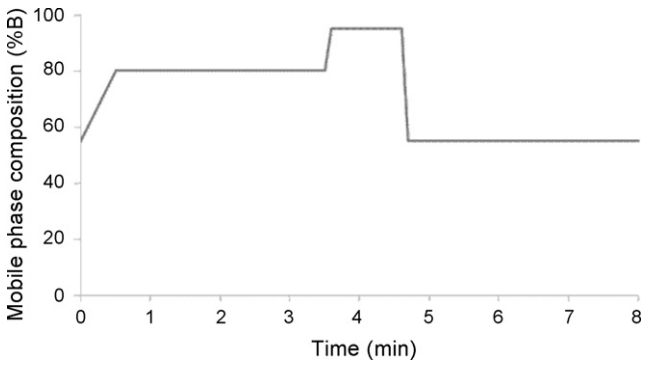

Fig. 3. HPLC gradient used in the LC–MS/MS-assay for vatalanib.

obtained. Analyte retention times (t_r 3.2 min) were sufficient to separate vatalanib and imatinib-$^{13}C,^{2}H_3$ from endogenous interferences.

3.3. Sample pre-treatment

Protein precipitation (PP) was used as sample pre-treatment, mainly because it is a fast and simple one-step procedure and the costs are very minor. However, PP lacks specificity and selectivity. For vatalanib a 3-fold signal reduction was observed when processed (PP with 100% acetonitrile) and non-processed samples were compared. This could be due to inclusion of the analyte during protein precipitation. To prevent loss of analyte a less strong precipitation fluid consisting of acetonitrile/methanol (1:1, v/v) was tested. This resulted in an increase in peak intensity with 20%. Liquid–liquid extraction with t-butyl methyl ether was tested, but the signal to noise ratio did not improve. Moreover, the sensitivity after protein precipitation was sufficient to quantify vatalanib in plasma within the therapeutic window. Additionally, the robustness of the sample pre-treatment with protein precipitation, determined by degree of variation of area ratio within repetitive measurements, was comparable to the robustness of the method with liquid–liquid extraction (CV%, 4.0 versus 5.0). There-

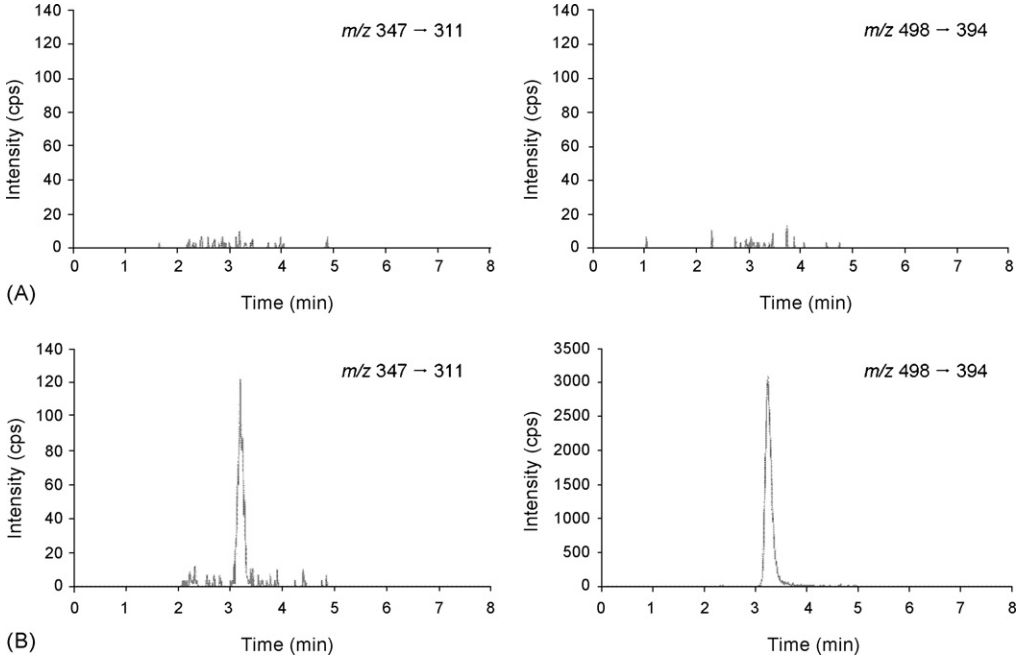

Fig. 4. Representive LC–MS/MS chromatograms of a blank human EDTA plasma sample (A1, vatalanib; A2, internal standard imatinib-$^{13}C,^{2}H_3$) and of a spiked human EDTA plasma sample at the LLQ level of 10 ng/mL (B1, vatalanib; B2, internal standard imatinib-$^{13}C,^{2}H_3$).

Table 1
Assay performance data for vatalanib.

Run	Nominal conc. (ng/mL)	Mean calculated conc. (ng/mL)	Inaccuracy (% dev.)	Precision (% CV)	No. of replicates
1	10.2	10.8	6.08	5.60	5
2	10.2	11.2	9.57	8.06	5
3	10.2	10.5	3.12	11.4	5
Inter-assay	10.2	10.8	6.25	8.38	15
1	20.5	20.5	−0.20	10.4	5
2	20.5	22.4	9.07	6.27	5
3	20.5	20.4	−0.39	7.69	5
Inter-assay	20.5	21.1	2.83	8.81	15
1	205	192	−6.44	2.94	5
2	205	188	−8.39	2.78	5
3	205	210	2.54	4.38	5
Inter-assay	205	197	−4.10	6.08	15
1	2050	2060	0.49	5.41	5
2	2050	2113	3.05	6.78	5
3	2050	2134	4.10	1.89	5
Inter-assay	2050	2101	2.51	4.79	15

Conc., concentration; dev, deviation; CV, coefficient of variation.

Table 2
Stability data for vatalanib and the internal standard imatinib-$^{13}C,^{2}H_3$.

Matrix	Conditions	Initial conc. (ng/mL)	Measured conc. (ng/mL)	Dev. (%)	CV (%)	No. of replicates
Vatalanib						
Plasma	Three freeze (−20 °C)/thaw cycles	19.8	20.3	2.18	4.31	3
Plasma	Three freeze (−20 °C)/thaw cycles	2160	2190	1.39	2.02	3
Plasma	Ambient, 6h	22.1	23.4	5.57	4.05	3
Plasma	Ambient, 6h	2113	2383	12.8	15.8	3
Final extract	2–8 °C, 7 days	20.7	20.9	1.29	7.71	3
Final extract	2–8 °C, 7 days	2177	2253	3.52	1.25	3
Final extract	Ambient, 48 h	20.9	21.5	10.7	3.82	3
Final extract	Ambient, 48 h	2120	2235	2.7	4.65	3
RR	Autosampler 4 °C, 48 h	19.8	20.0	0.67	5.02	3
RR	Autosampler 4 °C, 48 h	195	204	4.79	2.70	3
RR	Autosampler 4 °C, 48 h	2057	1997	−2.92	3.93	3
Stock solution (methanol)	Ambient, 6 h	0.512×10^6	0.497×10^6	−2.87	1.35	3
Imatinib-$^{13}C,^{13}H_3$						
Stock solution (methanol)	Ambient, 6 h	1.003×10^6	0.947×10^6	−5.57	2.00	3

Conc., concentration; dev., deviation; CV, coefficient of variation; RR, re-injection reproducibility.

fore, protein precipitation seems to be the most efficient sample pre-treatment for the analysis of vatalanib.

No deuterated internal standard of vatalanib was available. Therefore, stable isotopes of several tyrosine kinase inhibitors were tested as potential internal standards in this assay. Imatinib stable isotope co-eluted with vatalanib and corrected for variations in sample pre-treatment and analysis, and was therefore chosen as internal standard.

3.4. Validation

The assay was linear over the validated concentration range from 10 to 2500 ng/mL of vatalanib in human plasma. The lowest total bias and the most constant bias across the range were obtained using a weighting factor of $1/x^2$. Correlation coefficients (r^2) were at least 0.995. At all concentration levels the accuracies were within 95.9% and 106.3% with CV values less than 9.14%.

The intra- and inter-assay performance data are presented in Table 1. The intra-assay inaccuracies (% bias) for vatalanib in human EDTA plasma were within ±9.57% for all concentration levels. The intra-assay precisions (CV%) for vatalanib were less than 8.81% for all concentration levels. Samples above the ULQ (2500 ng/mL) were diluted 10 times with control drug-free human EDTA plasma. The intra-assay inaccuracy for diluted samples was 1.22% and the intra-assay precision was 3.23%. In conclusion, the validated range for vatalanib based on 50 μL human EDTA plasma is from 10.0 to 2500 ng/mL. When concentrations above 2500 ng/mL are expected, plasma samples can be diluted 10 times with control drug-free human EDTA plasma. Inaccuracies and precisions fulfilled the requirements [8].

In MRM chromatograms of six batches of control drug-free EDTA plasma no interference of endogenous compounds from plasma could be detected with the analyte or the internal standard. No co-eluting peaks >20% of the vatalanib peak area at the LLQ level were found and also no co-eluting peaks >5% of the internal standard imatinib-$^{13}C,^{3}H_2$ were detected. The deviations of the nominal concentration at the LLQ level were between −6.08% and 9.48% for vatalanib and were found to be acceptable.

When a sample of control drug-free human EDTA plasma was only processed with the internal standard imatinib-$^{13}C,^{3}H_2$, no peaks were detected at the retention time of vatalanib. Additionally, when a sample with vatalanib at ULQ level was processed without internal standard, no peaks were detected at the retention time of imatinib-$^{13}C,^{3}H_2$. In conclusion, no cross-analyte/internal standard interference was detected.

The mean matrix effect detected for vatalanib was an enhancement of 4.27% (range 2.91–6.59%). For vatalanib the mean PP recovery was 77.8% and the mean total recovery was 82.7%.

During development of the chromatographic system, different gradient systems were tested. Gradient elution with an increase in the amount of modifier by a quick stepwise gradient to 95% of eluent B after elution of vatalanib was chosen and the run time was

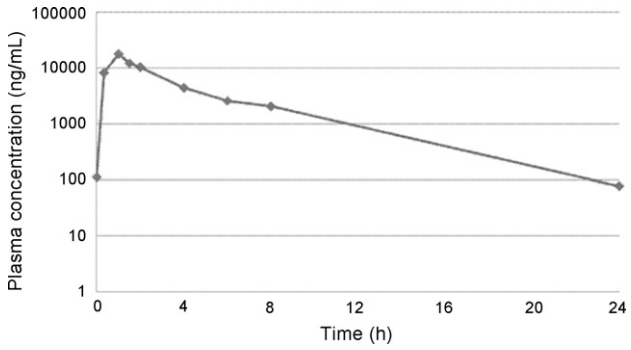

Fig. 5. Representative concentration versus time profile of vatalanib in a patient after one oral dose of vatalanib (750 mg).

set to 8 min to diminish carry-over due to a memory effect on the HPLC column. During validation of the present system no carry-over was experienced since no interfering peaks were detected in processed blank samples injected after an ULQ sample.

The stability data for vatalanib are represented in Table 2. Vatalanib is stable in human EDTA plasma for at least three freeze (−20 °C)/thaw cycles and in human EDTA plasma at ambient temperatures up to 6 h. Besides, vatalanib is stable in the final extract at nominally 2–8 °C up to 7 days. Re-injection reproducibility was established and an analytical run can be re-injected after at least 48 h of storage in the autosampler at 4 °C. Assessment of long-term stability of vatalanib in plasma and in stock solutions is still ongoing.

3.5. Application of assay in patient blood samples

The validated vatalanib assay was used to support a phase I pharmacokinetic trial of vatalanib in patients with advanced solid tumors [11]. Plasma samples were collected and thereafter processed and analyzed by the methods described in this report. All samples were stored at −70 °C until analysis. Because high concentrations were expected at the time points directly after administration of vatalanib, these plasma samples were diluted 10 times with control human EDTA plasma (50 μL plasma sample with 450 μL control human EDTA plasma) before processing. A representative plasma concentration–time profile for vatalanib after oral administration (dose: 750 mg once daily) is depicted in Fig. 5. The mean terminal half-life of vatalanib was calculated to be 5 h. Even 24 h after administration, the vatalanib plasma concentrations are far above the LLQ. These results demonstrate the applicability of the method in clinical pharmacokinetic studies.

4. Conclusion

We have developed and validated a fast LC–MS/MS method for the quantitative analysis of vatalanib in human plasma samples. Human EDTA plasma samples with vatalanib are pre-treated by protein precipitation with acetonitrile/methanol (1:1, v/v) and addition of internal standard imatinib-$^{13}C,^{3}H_2$. Chromatography is performed under alkaline conditions. A linear dynamic range from 10.0 to 2500 ng/mL was validated. Validation results show that the method is accurate and precise. The method is easy to perform and it has shown to be applicable in clinical pharmacological research. Additionally, since the required sample volume is relatively small (50 μL) and the method has a high sensitivity (S/N ≈ 10 at 10.2 ng/mL) it may also be useful for studies of vatalanib in which small sample volumes or low concentrations may be expected, such as other biological matrices.

References

[1] I.S. Moreira, P.A. Fernandes, M.J. Ramos, Anticancer Agents Med. Chem. 7 (2007) 223.
[2] D.R. Senger, W.L. Van de, L.F. Brown, J.A. Nagy, K.T. Yeo, T.K. Yeo, B. Berse, R.W. Jackman, A.M. Dvorak, H.F. Dvorak, Cancer Metastasis Rev. 12 (1993) 303.
[3] J.M. Wood, G. Bold, E. Buchdunger, R. Cozens, S. Ferrari, J. Frei, F. Hofmann, J. Mestan, H. Mett, T. O'Reilly, E. Persohn, J. Rosel, C. Schnell, D. Stover, A. Theuer, H. Towbin, F. Wenger, K. Woods-Cook, A. Menrad, G. Siemeister, M. Schirner, K.H. Thierauch, M.R. Schneider, J. Drevs, G. Martiny-Baron, F. Totzke, Cancer Res. 60 (2000) 2178.
[4] L.M. Jost, H.P. Gschwind, T. Jalava, Y. Wang, C. Guenther, C. Souppart, A. Rottmann, K. Denner, F. Waldmeier, G. Gross, E. Masson, D. Laurent, Drug Metab. Dispos. 34 (2006) 1817.
[5] E. Stokvis, H. Rosing, J.H. Beijnen, Mass Spectrom. Rev. 24 (2005) 887.
[6] B. Morgan, A.L. Thomas, J. Drevs, J. Hennig, M. Buchert, A. Jivan, M.A. Horsfield, K. Mross, H.A. Ball, L. Lee, W. Mietlowski, S. Fuxuis, C. Unger, K. O'Byrne, A. Henry, G.R. Cherryman, D. Laurent, M. Dugan, D. Marme, W.P. Steward, J. Clin. Oncol. 21 (2003) 3955.
[7] K. Mross, J. Drevs, M. Muller, M. Medinger, D. Marme, J. Hennig, B. Morgan, D. Lebwohl, E. Masson, Y.Y. Ho, C. Gunther, D. Laurent, C. Unger, Eur. J. Cancer 41 (2005) 1291.
[8] U.S. Food and Drug Administration: Centre for Drug Evaluation and Research. Guidance for Industry: Bioanalytical Method Validation. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatory Information/Guidances/UCM070107.pdf (accessed 1.5.01).
[9] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Pharm. Res. 24 (2007) 1962.
[10] E. Stokvis, H. Rosing, L. Lopez-Lazaro, I. Rodriguez, J.M. Jimeno, J.G. Supko, J.H. Schellens, J.H. Beijnen, J. Mass Spectrom. 37 (2002) 992.
[11] M.H. Langenberg, P.O. Witteveen, A.G. Lankheet, J. Clin. Oncol. 27 (2009) (Suppl; abstr. 2575).