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High-performance liquid chromatography method with ultraviolet detection for the quantification of the BCR-ABL inhibitor nilotinib (AMN107) in plasma, urine, culture medium and cell preparations

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Abstract

An isocratic and sensitive HPLC assay was developed allowing the determination of the new anticancer drug nilotinib (AMN107) in human plasma, urine, culture medium and cell samples. After protein precipitation with perchloric acid, AMN107 underwent an online enrichment using a Zirchrom-PBD precolumn, was separated on a Macherey-Nagel C18-HD column and finally quantified by UV-detection at 258 nm. The total run time is 25 min. The assay demonstrates linearity within a concentration range of 0.005–5.0 μg/ml in plasma ($r^2 = 0.9998$) and 0.1–10.0 μg/ml in urine ($r^2 = 0.9913$). The intra-day precision expressed as coefficients of variation ranged depending on the spiked concentration between 1.27–9.23% in plasma and 1.77–3.29% in urine, respectively. The coefficients of variation of inter-day precision was lower than 10%. Limit of detection was 0.002 μg/ml in plasma and 0.01 μg/ml in urine. The described method is stable, simple, economic and is routinely used for in vivo and in vitro pharmacokinetic studies of AMN107.

Keywords: Nilotinib; AMN107; Imatinb; Chronic myelogenous leukaemia; Tyrosine kinase; BCR-ABL mutant; HPLC; Intracellular concentration

1. Introduction

The deregulation of protein kinases has been identified to play a key role in the molecular pathogenesis of human cancers, such as chronic myelogenous leukaemia (CML), and also in solid tumors. CML is characterized by a reciprocal *t*(9;22) chromosomal translocation (Philadelphia chromosome) which results in a constitutive activation of the ABL tyrosine kinase. The aminopyrimidine imatinib (STI571, Gleevec) a potent ATP-competitive inhibitor of the BCR-ABL kinase, was the start for a new era of molecular targeted therapy. High rates of >95% response in patients with chronic phase CML were achieved. Despite the fact that the majority of patients receiving imatinib respond to treatment, relapse occurs in a certain subset of patients with advanced disease mostly caused by point mutations leading to a reduced affinity of imatinib to the ATP binding domain of the BCR-ABL protein and a reactivation of BCR-ABL kinase activity [1–11].

The new successor substance of imatinib is nilotinib (AMN107) (Fig. 1). For oral administration, nilotinib is available in capsules as the monohydrate form of the monohydrochloride salt. The mole weight is 583.99 g/mol. Nilotinib is an aminopyrimidine as is imatinib and was rationally designed using structural information from the X-ray structure of the imatinib–Abl complex, with a key feature being the replacement of the *N*-methylpiperazinyl group of imatinib. Like imatinib, AMN107 binds to an inactive conformation of the BCR-ABL enzyme by occupying a pocket in the ATP binding site. In studies with cell-lines expressing BCR-ABL, AMN107 displays more than 20-fold higher potency than imatinib in blocking proliferation. Weisberg et al. were able to show that the majority of 33 tested imatinib resistant BCR-ABL mutants were sensitive to AMN107 [15]. While the inhibition of the BCR-ABL tyrosine kinase activity by AMN107 in vitro is 10- to 50-fold more potent compared with imatinib, the potency against the c-kit (CD117)

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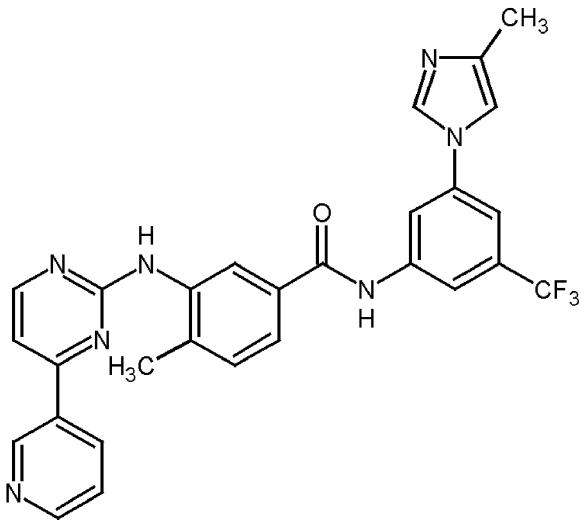

Fig. 1. Chemical structure of AMN107.

and platelet-derived growth factor receptor (PDGF-R) kinases are very similar to those of imatinib [12–17].

In clinical dose-escalation studies in imatinib-resistant CML as well as Ph+ ALL patients received 50–1200 mg AMN107 daily p.o. Hematologic response rates were achieved in 92% of patients with CML in chronic phase, 75% in accelerated phase and in 39% of patients in blast crisis while only 2 out of 13 patients with Ph+ ALL responded [18].

Based on validated HPLC methods, the pharmacokinetics of Gleevec is meanwhile well investigated, while only rudimentary informations on the pharmacokinetics of AMN107 exist to date [18–29]. Guetens et al. have recently presented a new HPLC method for simultaneous measurement of imatinib and AMN107 in plasma and cultured tumor cells, demonstrating the need for a simple analytical method for this new compound [32].

We here describe the development and validation of an alternative HPLC method for the determination of AMN107 in human plasma, urine and tumor cell lines. The method can be used as a tool to investigate AMN107 pharmacokinetics in the context of clinical studies and is helpful for basic research of e.g. cellular uptake of AMN107.

2. Experimental

2.1. Reagents, cell incubation and materials

AMN107 was kindly provided by Novartis Pharma (Basel, Switzerland). Powder was dissolved and stored at −20 °C as 1 mg/ml stock solution in ethanol (EtOH) and diluted in RPMI medium (Roswell Park Memorial Institute), plasma or urine for use. High purity solvents and salts were purchased from Merck (Darmstadt, Germany). Blank human plasma (anticoagulant: disodium EDTA) and urine samples were taken from healthy volunteers. K562, HL-60 and U87-MG cells lines for intracellular assay validation were obtained from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Braunschweig, Germany) and were grown in RPMI-1640 medium (Biochrom AG, Berlin, Germany) containing 10% fetal bovine serum, 1% penicillin/streptomycin in an atmosphere of 5% CO_2 at 37 °C.

2.2. HPLC apparatus

The chromatographic system consisted of a Waters 626 LC system, Waters 501 HPLC pump, Waters 717plus autosampler, Waters CHM column oven with TCM temperature controller (Waters, Eschborn, Germany), a Shimadzu UV spectrometric detector SPD-6A (Shimadzu, Duisburg, Germany) adjusted to a wavelength of 258 nm, a Rheodyne 7740-001 switching valve (Besta HPLC-Technik, Wilhelmsfeld, Germany) for automatic enrichment (Fig. 2). The authenticity proof of the compound was done with a GAT-PHD 601 diode-array detector (Gamma Analysentechnik GmbH, Bremerhaven, Germany). The GINA-Star software (Raytest, Germany) was applied for data acquisition, evaluation and integration of chromatograms.

2.3. Chromatography

Analytical separations were conducted using a 125 mm × 2 mm (I.D.), 3.5 μm, 100 Å, Macherey-Nagel Nucleosil C_{18} HD column (Macherey-Nagel GmbH & Co. KG, Düren, Germany) at a temperature of 40 °C. Since the enrichment process provides a high sample quality, a precolumn was not necessary. A 10 mm × 2 mm (I.D.) ZirChrom-PBD guard column was used as the enrichment column. Flow was set to 0.25 ml/min in the analytical part and to 1.6 ml/min in the enrichment part. The analytical eluent was composed of acetonitrile + 0.05 M KH_2PO_4 buffer (37:63, v/v, pH 4.03) containing 0.5% acetic acid. The enrichment eluent consisted of methanol + 0.02 M KH_2PO_4/0.02 M KH_2PO_4 (15:85, v/v, pH 7.18). Both solutions were degassed over 10 min with helium gas. Quantification was performed by using ultraviolet detection at 258 nm. Sample injection volume was 200 μl; after 50 injections of plasma, urine or cell samples the enrichment column was routinely replaced, even in cases of a high quality peak shape. As shown in Fig. 2, analysis started after injection in position A, with enrichment over 6 min. Thereafter, the valve was automatically switched to position B, whereby enrichment column was applied to the analytical part of the system and AMN107 was eluted to the analytical column and subsequently to the detector. The total run time, including enrichment period and re-equilibration over 2 min, was 25 min.

2.4. Quantification of AMN107

For quantification of plasma, urine, cerebrospinal fluid, RPMI and cells samples, calibration standards were prepared as a pool by adding an appropriate aliquot of the AMN107 spiking stock solution to the respective blank sample. Subsequently the standards were portioned out in 10 samples for each concentration and separately prepared and measured. For the standard curves, the concentrations in human plasma were 5, 10, 25, 50, 100, 250, 500, 750, 2500 and 5000 ng/ml, for urine 100, 500, 1000, 2000, 5000 and 10,000 ng/ml and for RPMI medium 100, 500 and 1000 ng/ml, respectively. Individual standard curves for differ-

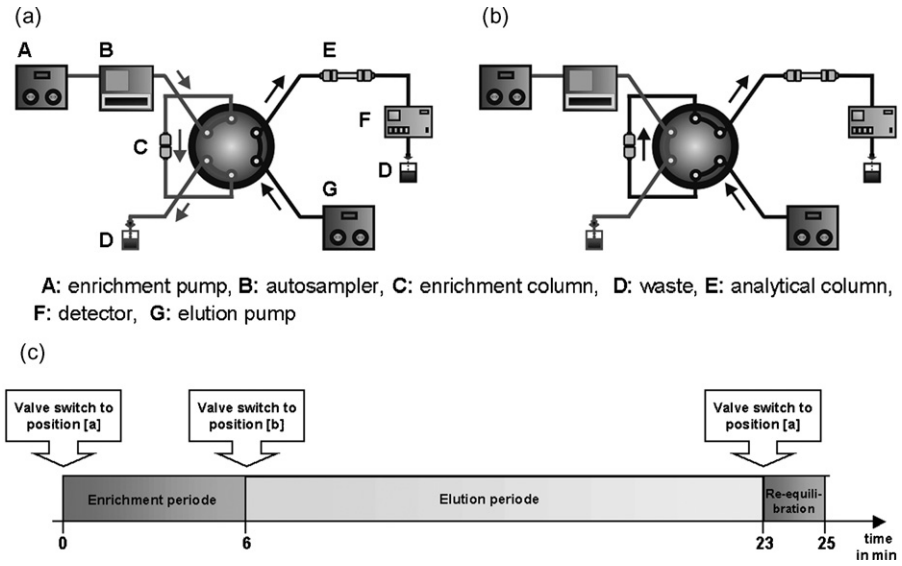

Fig. 2. Enrichment valve switching diagram. [a] Valve in enrichment position, enrichment column (C) in flow of enrichment system (bright arrows). [b] Valve in elution position, enrichment column (C) in flow of analytically system (dark arrows). [c] Graphical description of the time events of one several run.

ent types of samples were used since specific sample preparation procedures were applied to protein and non-protein containing samples leading to differences in recovery and injection volumina. Moreover, the usual concentration range in plasma and urine is not comparable (plasma 10–2000 ng/ml, urine 500–10000 ng/ml).

An unweighted, forced-through-zero, linear regression model was used to calculate the equation of relating peak areas and the concentrations of AMN107 in the calibrators.

2.5. Sample preparations

Three-hundred microlitres of plasma or other protein-containing samples (e.g. RPMI) were acidified by addition of 30 μl perchloric acid (85%) to precipitate the proteins. The mixtures were vortexed for 5 min and 170 μl of the so-called preparation solution (acetonitrile + 0.1 M KH_2PO_4, 40:60, v/v) were then added to enhance solubility of AMN107 and adjust the sample pH to 2.5. The samples were then re-vortexed for 10 min. After centrifugation for 5 min at 4000 × *g*, the supernatant (about 250 μl) was transferred into an autosampler vial and 200 μl were injected for quantification. Prepared samples were stored at 4 °C if they could not be immediately analysed. We found no significant degradation at the analyte concentration over a time period of 4 days (data not shown).

For the determination of non-protein containing samples like urine or cerebrospinal fluid, 200 μl of the sample were diluted with 200 μl of sample preparation solution, vortexed for 5 min and then centrifuged at 4000 × *g* for 5 min. The mixture (about 250 μl) was transferred into an autosampler vial and 100 μl was injected. The urine samples were stabile at equivalent conditions described above (data not shown).

To analyse in vitro intracellular substance levels, 5×10^6 HL-60 or K562 cells were incubated in RPMI 1640 medium with various drug concentrations for 5 h. After treatment, cell count was determined by using a Casy1 cell counter (Schärfe System GmbH, Reutlingen, Germany). Subsequently, the cells were centrifuged at 4000 × *g* for 10 min and supernatant was discarded. Collected cells were washed in 10 ml phosphate buffered saline (PBS), re-centrifuged and resuspended in 300 μl isotonic NaCl (medicinal purity). The further preparation and analysis of the cell samples was identical as for plasma samples (protein-containing samples).

2.6. Method application

The developed assay was routinely used for the quantification of AMN107 in samples of plasma from humans, receiving between 50 and 1200 mg AMN107 orally once daily or 400 and 600 mg twice daily. Blood samples were obtained from 29 patients over a duration of up to 7 months. Pharmacokinetic parameters were calculated by the "Topfit 2.0" computer software (Gustav Fischer Verlag, Stuttgart, Germany).

Moreover we used the described method for analysing intracellular AMN107 levels in different cell lines, depending on external growth stimulation or correlation with proliferation assays, respectively.

3. Assay validation

3.1. Linearity

The linearity of the method was determined over a concentration range between 5 and 5000 ng/ml in plasma samples and between 100 and 10000 ng/ml in urine samples, respectively. Calibration standards were freshly spiked by diluting stock solutions as a pool and subsequently divided into 10 samples. Each sample was prepared and analysed separately. The level of acceptance for the correlation coefficient was set at $r^2 > 0.99$.

3.2. Intra- and inter-day precision and accuracy

The intra-day precision and accuracy of the developed method was tested by analysing quality control (QC) samples of 10 different human plasma and 5 different urine concentrations. Ten estimations were made on each concentration and measured on one day. The total time of evaluation period was 4 months. Precision was calculated as mean of these 10 estimations and expressed as the coefficient of variation (CV) for each concentration separately. A precision that did not exceed 10% of the coefficient of variation for the higher concentrations and 15% for the lower limit of quantification was regarded as acceptable. The accuracy was accepted if the mean of the measured concentration ranged between 85 and 115% of the spiked concentration. The accuracy was described as relative percent deviation from the target value. The inter-day precision for human plasma was determined by 20 QC samples containing 200 ng/ml AMN107 over a time period of 5 months, for urine ten QC probes containing 1000 ng/ml within 10 days were analysed. Statistical values of precision and accuracy are expressed as CV of the 10 analysed QC samples. For evaluation of intra-day precision and accuracy in RPMI 1640 medium, three concentration levels were observed and parameters were calculated as described above. For the validation of intracellular measurement, a similar procedure as for plasma or urine matrices is not realizable, since it is impossible to spike directly the cells with an exact drug concentration. The model we used to determine the reliability of intracellular measurements consisted of three compartments, RPMI 1640 medium (1), cells (2) and due to wash step PBS (3). Since AMN107 is metabolised in liver cells via cytochrome CYP3A4 and not in HL-60, K562 or U87-MG cell lines, we could postulate that these model is a closed system, without any metabolism of the analyte [30]. Therefore, 50×10^6 cells were incubated in 50 ml RPMI 1640 medium containing 2500 ng/ml AMN107 over 5 h. The pretreatment AMN107 concentration in RPMI 1640 medium was analyzed 10 times separately. After incubation 50 ml RPMI 1640 medium, containing 50×10^6 suspended cells, were divided into 10 samples. Subsequently each sample was prepared and analysed separately. To demonstrate the efficiency of the preparation of cellular samples, after first sample preparation, the cell precipitates were dried and prepared again for three times (reextraction 1–3). In summary we determined compound concentration in pretreatment RPMI 1640 medium, posttreatment RPMI 1640 medium, cells, PBS wash solution and reextract 1, 2 and 3 (Table 4). We estimated precision and accuracy by summarizing the concentrations in all of these compartments [21,25].

Because of a non-linear cellular uptake of AMN107, we correlated the measured extracellular drug concentrations (analysed RPMI incubation concentration) with the analysed intracellular drug level by an second order exponential regression.

3.3. Specificity and identification

Specificity was evaluated by excluding co-eluting substances in 10 blank human plasma and urine samples from different healthy volunteers. Moreover, we analysed plasma samples from 29 patients drawn before starting treatment with AMN107 for co-eluting substances. In addition, we tested blank RPMI 1640 medium and three cell lines (HL-60, K562 and U87-MG) for co-eluting substances. For authentication of the analyte standard stock solution, patient plasma, spiked plasma and urine samples were scanned by using a diode-array spectrophotometer over a wavelength ranging from 200 to 360 nm.

3.4. Recovery in plasma and urine samples

The recovery was obtained from an amount of AMN107 added to and extracted from human plasma and urine compared with AMN107 measured in the pure stock solution. The tested concentrations were 10, 500, 4000 ng/ml in plasma and 75, 1000, 5000 ng/ml in urine, respectively. Each concentration level was determined with five samples and the aimed recovery was 80–120%.

3.5. Stability

The stability of AMN107 in plasma and urine was examined with quality standards containing 200 ng/ml and 1000 ng/ml after 72 h at room temperature, 7 days at +4 °C and 30 days at −20 °C with at least four freeze-thaw cycles, analyses were performed in duplicate. Stock solution stability in DMSO was tested for 3 months at +4 °C and 18 months at −20 °C.

4. Results

4.1. Linearity

The calibration curves of peak area versus concentration were found to be linear over the evaluated range from 5.0 to 5000 ng/ml in plasma and from 100 to 10000 ng/ml in urine (Fig. 3a and b). The linear regression coefficient in plasma was $r^2 = 0.9998$ with a slope of 1.4954 while in urine these values were $r^2 = 0.9913$ with a slope of 0.6008 due to the different sample preparations and injection volumina, respectively. Consequently, two different calibration curves were used for quantification of AMN107 plasma and urine samples. In contrast, the calibration curve obtained for RPMI 1640 medium was comparable with the plasma curve with a regression coefficient of $r^2 > 0.999$ and a slope of 1.4739. Therefore we used the plasma calibration curve for quantification of AMN107 in RPMI 1640 samples.

4.2. Specificity and identification

Specificity was tested by analysing 10 different plasma and urine samples from healthy volunteers. In these samples, no co-eluting peak to AMN107 could be detected. After spiking theses samples with 200 ng/ml (plasma) or 1000 ng/ml (urine) AMN107, a new peak with the typical retention time (RT) of 8.23 min for AMN107 in standard samples could be detected.

Blank samples from 29 patients with Ph+ ALL, drawn before the first application of AMN107, gave no indication for an interfering substance at a mean retention time (RT) of 8.23 min

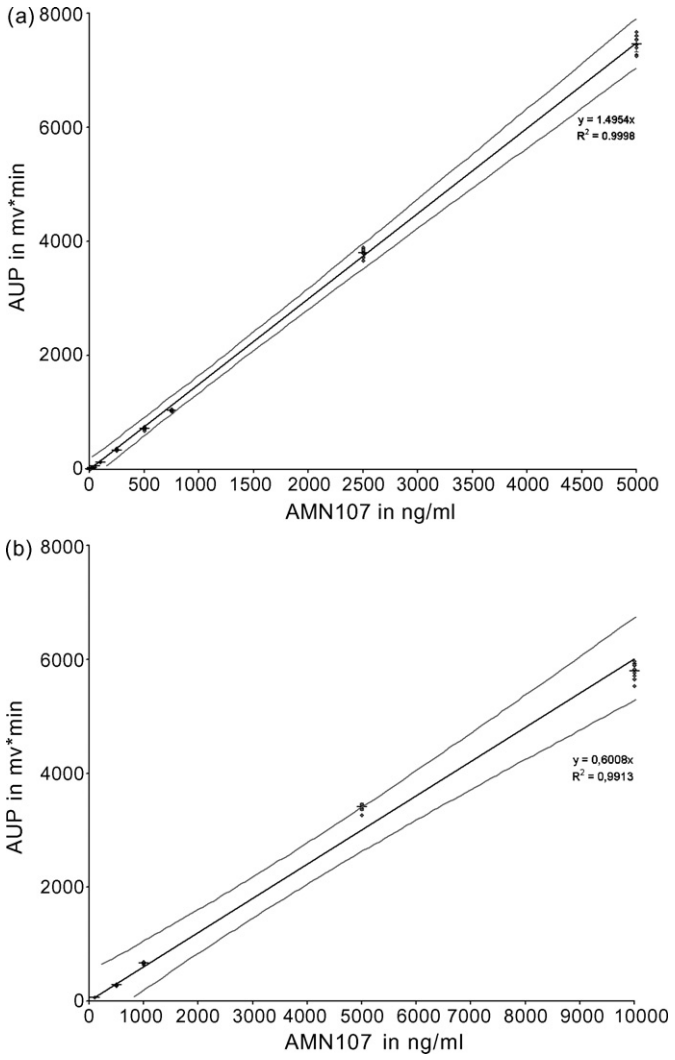

Fig. 3. Calibration curves and confidence limits ($a = 99.9999\%$, $b = 95\%$) for AMN107 in [a] plasma samples and [b] urine samples. Horizontal lines show the mean values, squares represent the respective measurement.

(SD ± 0.27 min, CV 3.33%). In all samples from these patients drawn after AMN107 application, a new peak with the RT of AMN107 could be detected.

Fig. 4a gives an example for a typical chromatogram of drug-free blank human plasma and urine while Fig. 4b demonstrates a chromatogram of spiked plasma. As depicted, no relevant interferences were detected at the RT of AMN107. Moreover, there were no co-eluting substances found in blank cell or RPMI 1640 samples.

Fig. 4d shows a chromatogram of a plasma sample from a patient treated with 400 mg AMN107 daily. The UV-spectra of spiked plasma and urine as well as patient plasma resulted in good comparable curves compared with the spectra taken from AMN107 stock solution (Fig. 5a–d).

4.3. Recovery in plasma and urine samples

The recovery was examined for low, medium and high concentration ranges in plasma and urine samples and was quite similar and consistent, independent of the concentration range. The average recovery of AMN107 was 101.12% (CV = 7.49%) in plasma and 94.65% (CV = 9.73%) in urine.

4.4. Intra- and inter-day precision and accuracy

The results obtained for intra-day precision and accuracy are summarized in Table 1 (plasma), Table 2 (urine) and Table 3 (RPMI 1640). The CV of intra-day precision for all evaluated concentrations including lower level of quantification (LLOQ) was less than 10%, which corresponds to the recommendations [31]. Values for accuracy, which were calculated as ratio of the mean value of measured concentration to spiked concentration, were also in the limit of acceptability. Table 4 demonstrates the results analysed by the evaluation of the intracellular AMN107 measurements. As shown, the overall recovery from these experiments was nearly complete with an acceptable CV.

The inter-day precision in plasma samples ($n = 17$) was 5.49% and accuracy was 89.78%, the corresponding mean values for urine samples ($n = 10$) were 4.56% and 106.80%, respectively. Overall, the inter-day precisions and accuracies were not essentially different from the intra-day data.

4.5. Stability

AMN107 was shown to remain stable in plasma and urine samples for at least 72 h at room temperature, 7 days at +4 °C and 30 days at −20 °C. No degradation of standard stock solution was observed after 3 months at 4 °C and 18 months at −20 °C. Moreover the prepared plasma and urine extracts were stable for at least 72 h at +4 °C.

4.6. Limit of detection and lower limit of quantification

An signal larger than two to three times of the base line noise is defined as limit of detection (LOD). For lower LLOQ a signal at least more largely than five times of the base line noise detected with a sufficient precision (<20%) and accuracy (80–120%) [31,32] is demanded. The LOD obtained for plasma samples was 2.0 ng/ml. The LLOQ was 5 ng/ml (Fig. 4c) with a precision of CV of 9.23% and accuracy of 89.96%. The signal to noise ratio was about 5.0. In urine, LOD was 10 ng/ml, however LLOQ was set to 100 ng/ml since the expected concentrations in patient urine, as approximated from animal results, are expected to be higher than 100 ng/ml.

4.7. Assay applications

The method was used for determination of AMN107 in plasma samples and various cellular applications. Fig. 6 shows mean concentration–time curves obtained from three patients with Ph+ ALL in the first 24 h after the first dose of AMN107 was taken (1 × 600 mg p.o.). AMN107 was absorbed quite slowly with a mean peak concentration of 1800 ng/ml achieved after 4.4 h. The mean elimination time ($t_{1/2}$) in these patients was 26 h. The area under the curve (AUC) calculated by using a linear two compartment model was 50 μg h/ml. A complete description of

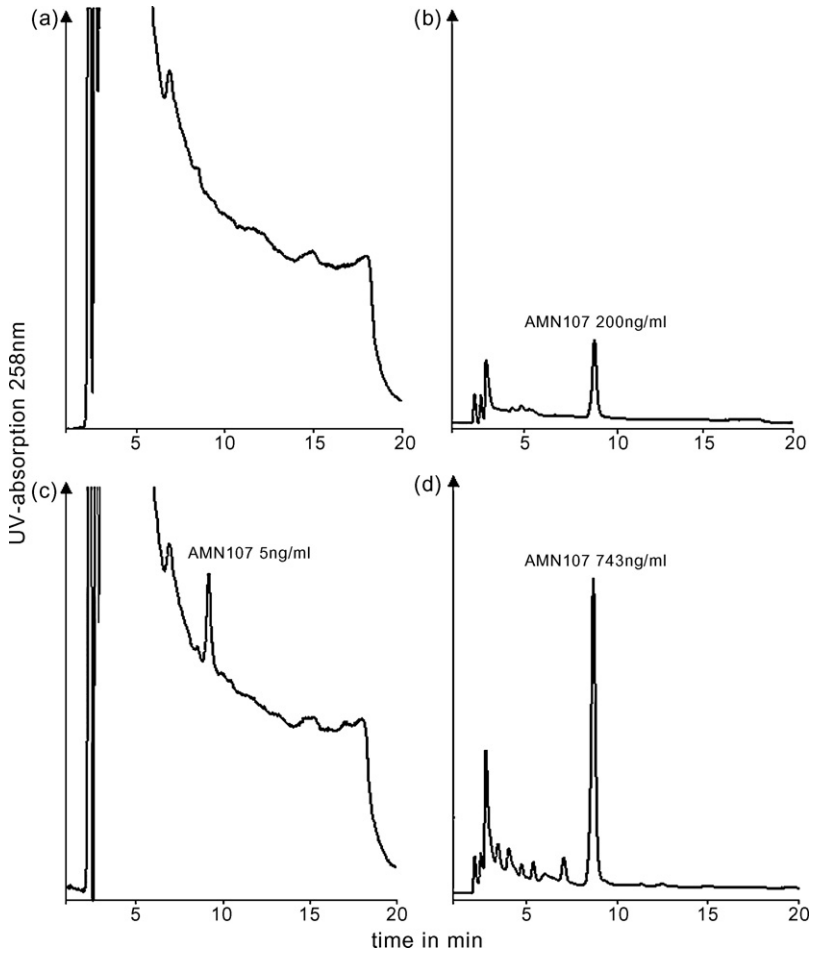

Fig. 4. Representative chromatograms of [a] blank human plasma; [b] spiked plasma with 200 ng/ml AMN107; [c] spiked plasma with 5 ng/ml AMN107; and [d] patient plasma under treatment with AMN107 containing 743 ng/ml AMN107.

Table 1
Intra-day assay precision in plasma samples

Spiked concentration (ng/ml)	Number of assays	Measured concentration mean (ng/ml)	SD (ng/ml)	CV (% precision)	Accuracy (%)
5000	10	4990.3	89.9	1.8	0.2
2500	10	2542.3	47.6	1.9	1.69
750	10	696.0	9.6	1.4	7.2
500	10	482.2	15.1	3.1	3.6
250	10	228.3	8.8	3.9	8.7
100	10	88.2	1.1	1.3	11.8
50	10	43.3	0.9	2.1	13.5
20	10	20.6	0.6	2.8	3.1
10	10	10.1	0.7	6.9	1.1
5	10	4.5	0.4	9.2	10.0

Table 2
Intra-day assay precision in urine samples

Spiked concentration (ng/ml)	Number of assays	Measured concentration mean (ng/ml)	SD (ng/ml)	CV (% precision)	Accuracy (%)
10,000	10	9649.0	227.8	2.4	3.5
5,000	10	5680.2	100.6	1.8	13.6
1,000	10	1113.0	22.3	2.0	11.3
500	10	480.1	15.8	3.3	4.0
100	10	106.7	3.0	2.8	6.7

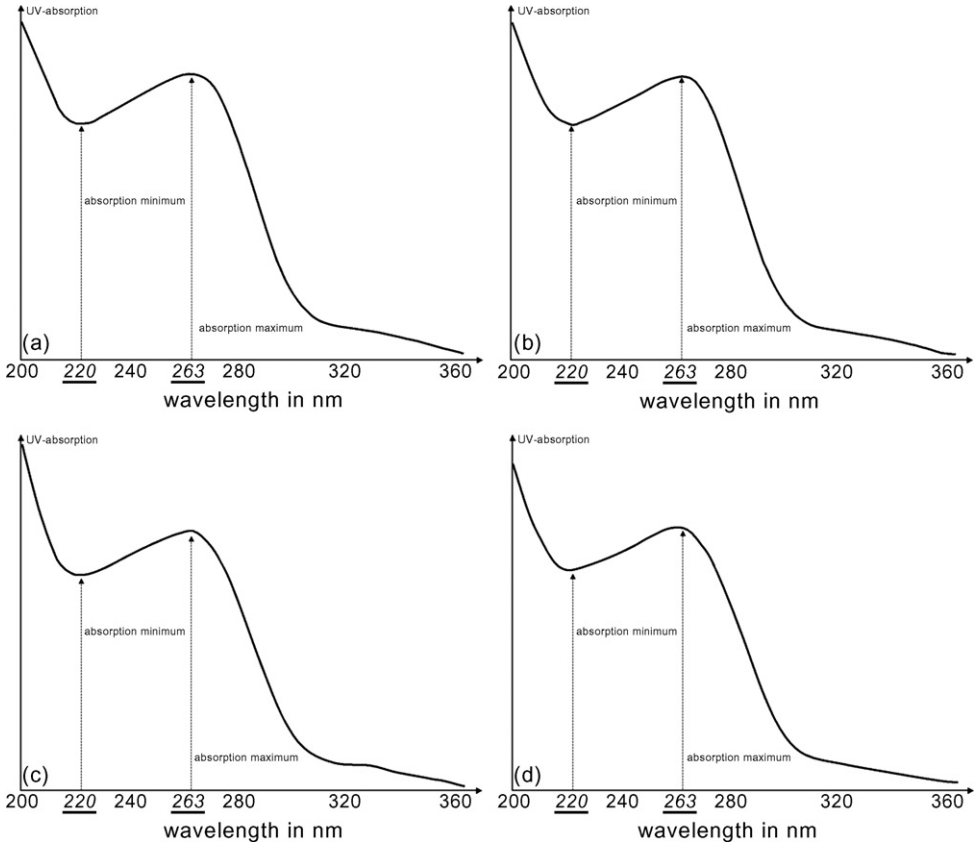

Fig. 5. UV-scan of AMN107 in different samples. [a] Aqueous standard solution. [b] Spiked plasma sample. [c] Patient plasma sample. [d] Spiked urine sample.

the pharmacokinetic results of this study involving all analysed patients data will be reported separately.

The dose-dependend intracellular concentration of AMN107 in HL-60 cell lines was determined in triplicates ($3 \times 5 \times 10^6$ cells in 5 ml RPMI 1640 medium) for each concentration level and repeated three times on different days (Fig. 7). Data thus obtained demonstrate a good repeatability of dose dependent cellular uptake of AMN107 in HL-60 cells within the examined time period of 7 days (the CVs were between 22.02% at lowest and 11.01% at highest concentration). The method of intracellular AMN107 quantification was transferred to K562 leukemia cell lines and U87-MG Glioblastoma multiforme cell

Table 3
Summary of intra-day assay precision in RPMI 1640 samples

Spiked concentration (ng/ml)	Number of assays	Measured concentration mean (ng/ml)	SD (ng/ml)	CV (% precision)	Accuracy (%)
1000	10	999.1	24.5	2.5	0.1
500	10	500.8	9.4	1.9	0.2
100	10	105.2	2.2	2.1	5.2

Table 4
Intra-day assay precision in the intracellular model; 50 × 106 HL-60 were incubated for 5 h in 50 ml RPMI 1640 medium spiked with 2500 ng/ml AMN107, then divided in 10 aliquots and analysed independently (details described in Section 3.2)

	Mean ($n = 10$) (ng absolute)	SD (ng absolute)	CV (% precision)	Compartment quantity (%)
Analysed RPMI before incubation	12411.9	377.9	3.0	100
Cellular AMN107	881.3	23.0	2.6	8.5
RPMI after incubation	9029.5	269.0	3.0	72.8
Washing solution	241.0	18.2	7.6	1.9
Reextraction 1	36.7	1.2	3.3	0.3
Reextraction 2	1.4	0.1	8.8	0.0
Reextraction 3	0.0	0.0	n.a.	0.0
Sum of AMN107 in all compartments (%)				83.5

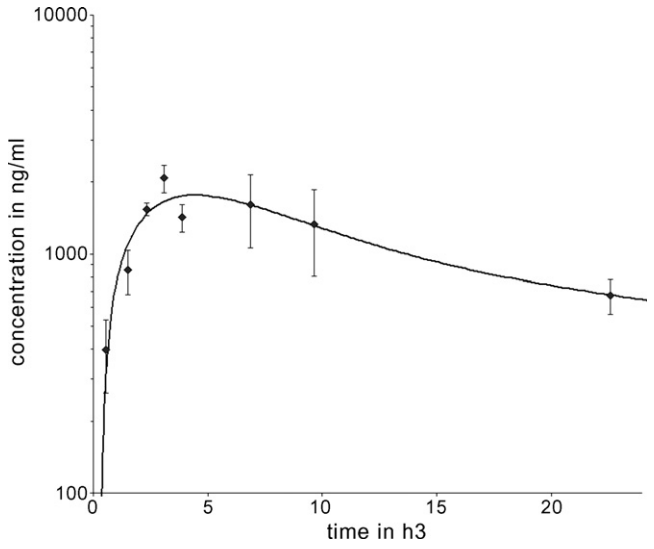

Fig. 6. Concentration–time-curve for AMN107: the data points represent the mean plasma levels of three patients after first drug intake of 600 mg. Vertical bars represents the standard deviation.

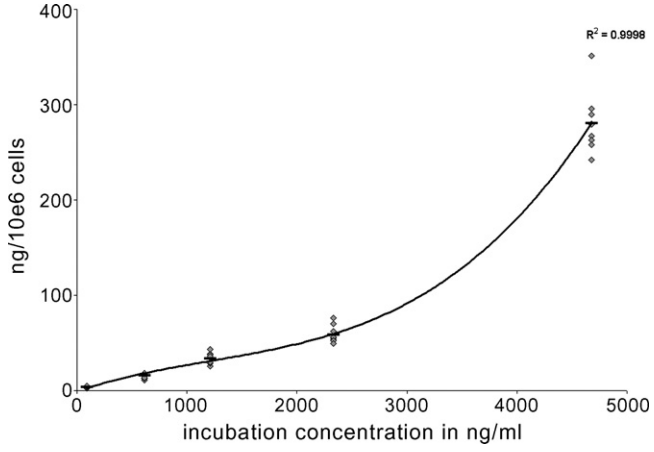

Fig. 7. Intracellular uptake of AMN107 in HL-60 cells: regression of AMN107 per 10^6 cells after 5 h incubation vs. pretreatment concentration of AMN107 in RPMI 1640 medium. Horizontal lines show the mean values, squares represent the respective measurement.

lines and cellular uptake was also investigated (data not shown). The detailed results from cellular uptake and drug interaction experiments will be reported separately.

5. Conclusion

We have developed an easily adaptable and economic HPLC method for the analysis of AMN107. No extensive sample preparation, derivatization or expensive and complex instrumentation like mass spectrometry is needed. The assay is routinely suitable for studying the pharmacokinetic profile of AMN107 in patients with cancer as well as for in vitro experiments. The criteria for linearity, specificity, precision, accuracy, recovery and stability have been proven to be within the recommendations of the FDA guidelines for validation of bioanalytical methods [31]. The method reaches the sensitivity required for quantification of AMN107 in human plasma up to concentrations of 5 ng/ml. Nearly all to date measured plasma samples show concentration levels greater than 100 ng/ml. A recently published UV-detection based HPLC-Method from Guetens et al. has the advantage of measuring AMN107 and imatinib simultaneously but reach a LOD of only 50 ng/ml [32].

For urine samples, we estimate concentrations of the compound far above 100 ng/ml; therefore, we expect the method to be sufficient for the analysis of clinical urine samples too. Moreover, the satisfying LLOQ of the method implicates that most cerebrospinal fluid samples with an estimated concentration between 10 and 100 ng/ml (from animal experiments) should also be detectable.

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