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Bioanalytical method for the quantification of sunitinib and its *n*-desethyl metabolite SU12662 in human plasma by ultra performance liquid chromatography/tandem triple-quadrupole mass spectrometry

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ABSTRACT

A rapid and sensitive ultra performance liquid chromatography/tandem mass spectrometry (UPLC–MS/MS) method has been developed and validated for the quantitative determination of sunitinib and its *n*-desethyl metabolite SU12662, in 100 μl aliquots of human potassium EDTA plasma with deuterated sunitinib as internal standard. As sunitinib was found to be extremely sensitive to light causing rapid conversion of the Z (*cis*)-isomer to the E (*trans*)-isomer, the sample extraction and cleaning-up were performed under sodium-light and in amber vials. The extraction involved a simple liquid–liquid extraction with *tert*-butyl methyl ether. Chromatographic separations were achieved on an Aquity UPLC® BEH C_{18} 1.7 μm, 2.1 mm × 50 mm column eluted at a flow rate of 0.250 ml/min on a gradient of acetonitrile. The overall cycle time of the method was 4 min, with elution times of 1.05, 1.43, 0.95, and 1.34 min, for the E (*trans*)- and Z (*cis*)-isomers of sunitinib and the E (*trans*)- and Z (*cis*)-isomers of SU12662, respectively. The multiple reaction monitoring transitions were set at 399 > 326 (*m*/*z*), at 371 > 283 (*m*/*z*) and at 409 > 326 (*m*/*z*) for sunitinib, SU12662 and the internal standard, respectively. The calibration curves were linear over the range of 0.200 to 50.0 ng/ml with the lower limit of quantitation validated at 0.200 ng/ml for both sunitinib and SU12662. The within-run and between-run precisions were within 11.7%, while the accuracy ranged from 90.5 to 106.8%.

1. Introduction

In recent years, numerous proteins and biochemical pathways, which play a crucial role in the pathogenesis of solid malignancies, have been identified. Many of these tumor-driving factors function as kinases. Compounds inhibiting the kinase activity of these proteins, thereby displaying anti-tumor activity, form an important novel class of anti-tumor agents, for which the value has already indisputably been proven in several tumor types.

Of this class of kinase inhibitors, sunitinib is one of the first, which is widely explored. It targets the kinase domains of several tumor-driving proteins including c-kit, the vascular endothelial growth factor receptors (VEGF-R), the platelet-derived growth factor receptors (PDGF-R), RET, Flt-3, and several others [1]. Based on randomised studies, sunitinib is currently regarded standard second line therapy for patients with advanced gastro-intestinal stromal tumors (GIST) who failed on imatinib [2] and is standard first line treatment for patients with advanced renal cell carcinoma (RCC), clear cell subtype [3]. Furthermore, sunitinib is explored for its anti-tumor activity in many other tumor types as single agent, but also in combination with other anti-tumor compounds.

As is the case for other anti-tumor agents, a dose–effect association probably also exists for kinase inhibitors [4]. With respect to sunitinib, it appeared that higher area under the plasma concentration time curves (AUC) of sunitinib and its active metabolite SU12662 are associated with better outcomes in RCC patients in terms of response rates, progression-free survival, and overall survival [5]. Higher doses of sunitinib are however frequently accompanied by unacceptable toxicities necessitating dose reductions and interruptions [6]. As there are considerable inter- and intrapatient variations in plasma levels of sunitinib and its metabolites, there is a high need for assays reliably determining sunitinib levels. Such assays are mandatory for a more individualised treatment approach of patients, thereby avoiding over- or under-treatment, and are essential to assess the interaction of sunitinib with other drugs.

In view of the evaluation of the pharmacokinetics of sunitinib in clinical studies, a bioanalytical method for sunitinib and its *n*-desethyl metabolite SU12662 in human potassium EDTA plasma needed to be developed and validated. Only a few analytical

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methods, all based on LC–MS/MS, have been described [7–11]. However, only two methods are of interest for pharmacokinetic laboratories, as the methods are described in detail. Minkin et al. [11] published a well-validated method for sunitinib in human plasma. Aliquots of 0.2 ml of plasma were processed by liquid–liquid extraction with 4 ml *tert*-butyl methyl ether. The LLQ was validated at 0.2 ng/ml. The method, however, lacks the ability to quantitate the active metabolite SU12662 and uses a non-deuterated internal standard. The latter potentially leads to ionization related drawbacks during the analysis of study samples, as we observed during our validation. Haznedar et al. [10] briefly describes two methods for the quantitation of sunitinib and SU12662 in rat plasma, following protein precipitation of 50 μl aliquots of plasma with either 2 ml acetonitrile or 500 μl methanol. The LLQs were validated at 0.1 and 0.07 ng/ml, respectively.

Sunitinib is supposed to be photosensitive in solutions, however, the literature is scarce and in both publications mentioning this phenomenon [10,11], an appropriate investigation of the light sensitivity of sunitinib is lacking. Zhao et al. [12] showed that semaxinib, a compound structurally closely related to sunitinib, is photosensitive too. In solid form semaxinib only exists as the Z (*cis*)-isomer. However, in solution the compound exists in two stereoisomeric forms known as the Z (*cis*) or E (*trans*)-isomer around the double bond between 2-oxindole and the pyrrole ring. The thermodynamically stable Z (*cis*)-isomer of semaxinib and related compounds can spontaneously convert to its E (*trans*)-isomer when exposed to light. The E (*trans*)-isomer is not stable in solution and reverts to the Z (*cis*)-isomer depending upon the exact conditions such as pH and protection from light [12].

Here, we report on the development and validation of a bioanalytical method for the simultaneous determination of sunitinib and its active metabolite SU12662 in human potassium EDTA plasma and we describe the influence of several light sources on the isomeric conversions. The method has been validated in agreement with the Guidance for Industry, Bioanalytical Method Validation, as specified by the Food and Drug Administration (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf).

2. Experimental

2.1. Chemicals

Sunitinib, *n*-desethyl sunitinib and the deuterated internal standard sunitinib-d10 were obtained from Toronto Research Chemicals (North York, ON, Canada). All chemicals were of analytical grade or higher. Acetonitrile, methanol and water were purchased from Biosolve BV (Valkenswaard, The Netherlands). Dimethylsulphoxide and *tert*-butyl methyl ether were from Sigma–Aldrich (Zwijndrecht, The Netherlands). Formic acid was obtained from J.T. Baker (Deventer, The Netherlands) and 2-propanol from Merck (Darmstadt, Germany). Blank potassium EDTA plasma was purchased from Biological Specialty Corporation (Colmar, PA).

2.2. Preparation of stock solutions, calibration standards and quality control samples

Sunitinib and *n*-desethyl sunitinib stock solutions were prepared at 1 mg/ml free base in dimethylsulphoxide. Stock solutions were aliquotted and stored at $T < -70\,^{\circ}C$. Separate stock solutions were prepared for the construction of the calibration curve standards and the pools of quality control samples. The internal standard stock solution was prepared at 1 mg/ml free base in dimethylsulphoxide, which subsequently was aliquotted and stored at $T < -70\,^{\circ}C$. Aliquots of 10 μl were 10,000-fold diluted in acetonitrile, resulting in an internal standard solution containing 100 ng/ml sunitinib-d10 as free base, which was stored at $T < 8\,^{\circ}C$ for a maximum of 3 months.

Calibration standards were prepared freshly on the day of analysis, in duplicate, by addition of 25 μl aliquots of appropriate dilutions of sunitinib and *n*-desethyl sunitinib stock solution in acetonitrile/DMSO (1:1, v/v) to 475 μl aliquots of human potassium EDTA plasma at the following concentrations: 0.200, 0.500, 5.00, 10.0, 20.0, 30.0, 45.0, and 50.0 ng/ml as free base.

Five pools of QC samples were prepared in human potassium EDTA plasma at concentrations of 0.200 ng/ml (lower limit of quantitation, LLQ), 0.600 ng/ml (QC-Low), 25.0 ng/ml (QC-Middle), 40.0 ng/ml (QC-High) and 150 ng/ml (QC-Diluted). QC-Diluted was processed after a 20-fold dilution in blank human potassium EDTA plasma. Pools of QC samples were aliquotted and stored at $T < -70\,^{\circ}C$ upon processing.

2.3. Plasma sample preparation

Aliquots of 50 μl of internal standard solution were added to 100 μl of plasma samples in 2 ml amber microcentrifuge tubes. Subsequently 150 μl aliquots of acetonitrile were added, followed by 1 ml aliquots of *tert*-butyl methyl ether. Hereafter, the samples were vigorously mixed for 5 min and then centrifuged at 18,000 × *g* at ambient temperature for 10 min. Aliquots of 950 μl of the organic phase were transferred into 1.5-ml amber microcentrifuge tubes and evaporated under nitrogen at $T = 70\,^{\circ}C$. The residues were resuspended in 100 μl aliquots of acetonitrile/water/formic acid (20:80:0.1, v/v/v) by ultrasonification. After centrifugation for 5 min at 18,000 × *g*, the supernatants were transferred into 350 μl 96-well plates, which were placed into a light protected, chilled ($T = 10\,^{\circ}C$) autosampler, from which aliquots of 5 μl were injected onto the UPLC column.

2.4. Equipment

The UPLC–MS/MS system was composed of a Waters Aquity UPLC Sample Manager coupled to a Waters TQ Detector (Waters, Etten-Leur, The Netherlands). The MassLynx V4.1 SCN627 software package was used for the acquisition and processing of data. Quantification was performed using QuanLynx as implemented in the MassLynx software.

2.4.1. Chromatographic conditions

Analytes were separated on an Aquity UPLC® BEH C_{18} column 1.7 μm, 50 mm × 2.1 mm (Waters, Etten-Leur, The Netherlands) thermostatted at $T = 40\,^{\circ}C$. A gradient, at a flow rate of 0.250 ml/min, was achieved with mobile phase A, composed of water, acidified with 0.1% formic acid and mobile phase B, composed of acetonitrile acidified with 0.1% formic acid. Following a partial loop injection of 5 μl, a linear gradient separation was used, with 20–100% of mobile phase B from 0 to 3 min, then 100–20% of mobile phase B over 0.1 min, holding for 0.9 min for initial conditioning. The overall run time of the assay was 4 min. The needle wash solvent was composed of acetonitrile/methanol/water/2-propanol/formic acid (25:25:25:25:0.1, v/v/v/v/v). The column effluent was passed through the mass spectrometer and monitored.

2.4.2. Mass spectrometry

Tandem mass spectrometry was performed in the positive ion electrospray ionization mode. Mass transitions of *m*/*z* were optimized for sunitinib, SU12662, and sunitinib-d10 (IS) by infusion of the respective analytes in acetonitrile/water/0.1% formic acid (20:80:0.1, v/v/v) via combined infusion. Optimal MS settings were manually adjusted. The desolvation gas was set at 800 ml/h

(nitrogen). The ionspray voltage was kept at 1.50 kV and the cone voltage at 38 V for sunitinib and sunitinib-d10 (IS) and 35 V for SU12662, with a source temperature of $T = 120\,^{\circ}C$ and desolvation temperature of $T = 350\,^{\circ}C$. The dwell times were set at 50 ms and the inter-channel delay at 100 ms. Multiple reaction monitoring (MRM) mode was applied for the quantitation with the following parameters: m/z 399 > 326, collision energy at 22 eV for sunitinib, m/z 371 > 283, collision energy at 20 eV for SU12662 and m/z 409 > 326, collision energy at 20 eV for the IS. The collision cell pirani pressure was set at $\sim 5e^{-3}$ mbar (argon).

2.4.3. Quantitation

Calibration curves were generated using peak area ratios of sunitinib and SU12662 to internal standard sunitinib-d10 versus the known sunitinib and SU12662 concentrations with a linear regression equation of 1/concentration^2.

2.5. Light sensitivity of sunitinib and n-desethyl sunitinib

Prior to the validation, several experiments in which sunitinib and SU12662 were exposed to several light sources, were conducted to investigate the stability of the isomers during sample handling and preparation. In the final experiment, standard solutions at a concentration of 150 ng/ml in acetonitrile/water/formic acid (20:80:0.1, v/v/v) of the Z (*cis*)-isomer of both compounds were prepared under light protected conditions. Three sample sets were prepared using this standard solution. The first set was exposed for 4 h to sodium-light (584 nm), the second set was exposed to UV-light (254 nm) and the third set was exposed to daylight (~350–700 nm). These sets of standard solutions were analyzed by UPLC–MS/MS using the conditions described in the experimental sections.

2.6. Method validation

The UPLC–MS/MS method was validated in accordance with the Guidance for Industry, Bioanalytical Method Validation, as specified by the Food and Drug Administration (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf). The potential presence of endogenous contaminating compounds that may interfere with the analytical assay was determined by analyzing blank human potassium EDTA plasma samples of 10 different lots. The following substances were investigated for interference with the analytical method: aprepitant, dexamethasone, domperidon, granisetron, lorazepam, oxazepam, paracetamol, ranitidine and metoclopramide. All drugs have been dissolved/diluted in water to a concentration of 1 mg/ml followed by a 500-fold dilution in blank human potassium EDTA plasma (final concentrations 2 μg/ml). Aliquots of QC-Diluted (i.e. 150 ng/ml) have subsequently been diluted in the plasma containing the different drugs to yield final sunitinib and SU12662 concentrations of 0.600 and 40.0 ng/ml, which has been processed and compared to equal dilutions of QC-Diluted in blank human potassium EDTA plasma. In addition, to invest the potential interference of the validated bioanalytical method in a clinical study, in which intravenous ifosfamide is given without or in combination with oral sunitinib, aliquots of QC-Diluted have been diluted as described above in plasma samples collected from patients during and following the ifosfamide infusion in the absence of sunitinib. The patients gave written informed consent for blood collection and the local institutional review boards approved the clinical protocol, which was written in accordance with the declaration of Helsinki (see: http://www.wma.net/e/policy/b3.htm).

For the determination of the LLQ, blank human potassium EDTA plasma of 10 different donors was spiked at a concentration of 0.200 ng/ml for both compounds. Accuracy (ACC), within-run precision (WRP) and the between-run precision (BRP) were determined by analyzing five replicates of pools of LLQ and QC samples independently over a three-run period, with the calibration curve standards processed in duplicate. The ACC, WRP and BRP at the level of the LLQ and QC samples were calculated by one-way analysis of variance, using the run as the variable as described previously [13,14].

The evaluation of the matrix effect for both compounds was tested by comparing the MS/MS response of sunitinib and SU12662 levels spiked in triplicate in six lots of blank potassium EDTA plasma to the MS/MS responses of the analytes spiked in triplicate into blank plasma extracts as described recently [13].

Extraction recovery (RE) was determined by comparing the MS/MS response of sunitinib and SU12662 at the same concentration levels spiked in quintuplicate into blank human potassium EDTA plasma before extraction, to the MS/MS response of sunitinib and SU12662 spiked in triplicate into extracts of blank human potassium EDTA plasma, corrected for the evaporated volume of organic phase [13].

The stability of sunitinib and its *n*-desethyl metabolite SU12662 in human potassium EDTA plasma was tested with QC-Low and QC-High at ambient temperature for a period of 6 h and for three freeze–thaw cycles, in which the samples were thawed for at least 15 min before refreezing them for at least 18 h. The storage stability of processed samples in the autosampler was tested in triplicate at the concentration of QC-Low and QC-High in plasma. QC samples were processed in triplicate and repeatedly injected at different time points.

2.7. Application of method to clinical samples

To demonstrate the applicability of the validated bioanalytical method, blood samples were collected from a 29-year-old female patient with metastatic melanoma enrolled in a clinical phase I trial, in which oral sunitinib at a dose of 12.5 mg/daily was given in combination with ifosfamide administered as a 5-day continuous intravenous infusion at a dose of 1200 mg/m^2/day. Sunitinib dosing was started 7 days after the first ifosfamide infusion. A 5 ml blood sample was collected in the presence of potassium EDTA as anticoagulant pre-dose at day 7, prior to dosing every 3–4 days and daily during the second ifosfamide cycle. Blood samples were centrifuged within 15 min after collection for 10 min at 3000 × *g* at $T = 4\,^{\circ}C$. The plasma was stored

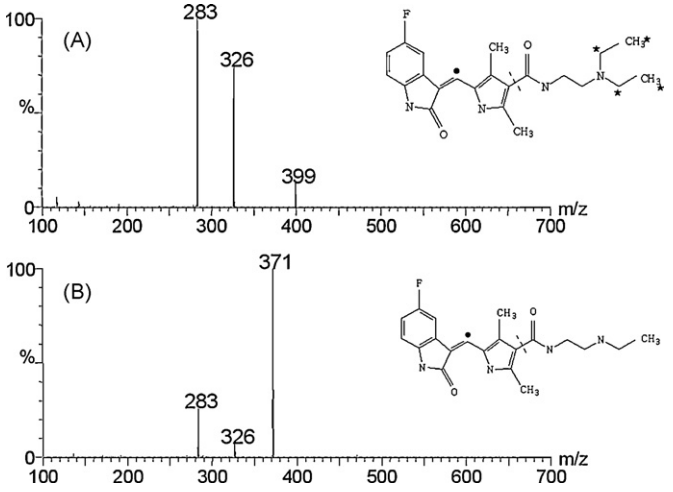

Fig. 1. Mass spectrum and analytical structures of sunitinib (A) and its *n*-desethyl metabolite SU12662 (B). The asterisks represent the deuterium atoms in the deuterated internal standard sunitinib-d10. The chiral centers are indicated by the dot.

at $T < -70\,^{\circ}C$, in vials wrapped with aluminium-foil, until analysis. The patient gave written informed consent and the local institutional review boards approved the clinical protocol, which was written in accordance with the declaration of Helsinki (see: http://www.wma.net/e/policy/b3.htm).

3. Results and discussion

3.1. LC–MS/MS conditions and method development

The sunitinib and SU12662 product ion spectra (Fig. 1) yield abundant product ions suitable for use in multiple reaction monitoring. The product ion at *m*/*z* 326 using collision energy of 22 eV was selected as the MRM ion for quantitation of sunitinib and its deuterated internal standard sunitinib-d10 (collision energy of 20 eV). For SU12662, the product ion at *m*/*z* 283 using collision energy of 20 eV was selected as the MRM ion for quantitation.

By applying a steep gradient, sunitinib and SU12662 were separated from early eluting hydrophilic, potentially response-suppressing, matrix components, while maintaining a relative short analysis time of 4 min, with sunitinib eluting at 1.4 min and SU12662 at 1.3 min (Fig. 2). Two additional minor peaks were observed in chromatograms due to the Z (*cis*)–E (*trans*) isomerization reaction. The retention time of the E (*trans*)-isomers of SU12662 and sunitinib were 0.95 and 1.06 min, respectively. As shown the E (*trans*)-isomers of both sunitinib and SU12662 were base-line separated from the respective Z (*cis*)-isomers (Fig. 2).

3.1.1. Light sensitivity of sunitinib and SU12662

In order to make use of the Z (*cis*)-isomer of sunitinib and SU12662 for the quantitation, the extent of conversion of the Z (*cis*)-isomer to the E (*trans*)-isomer under different light source conditions was studied. One set was exposed for 4 h to sodium-

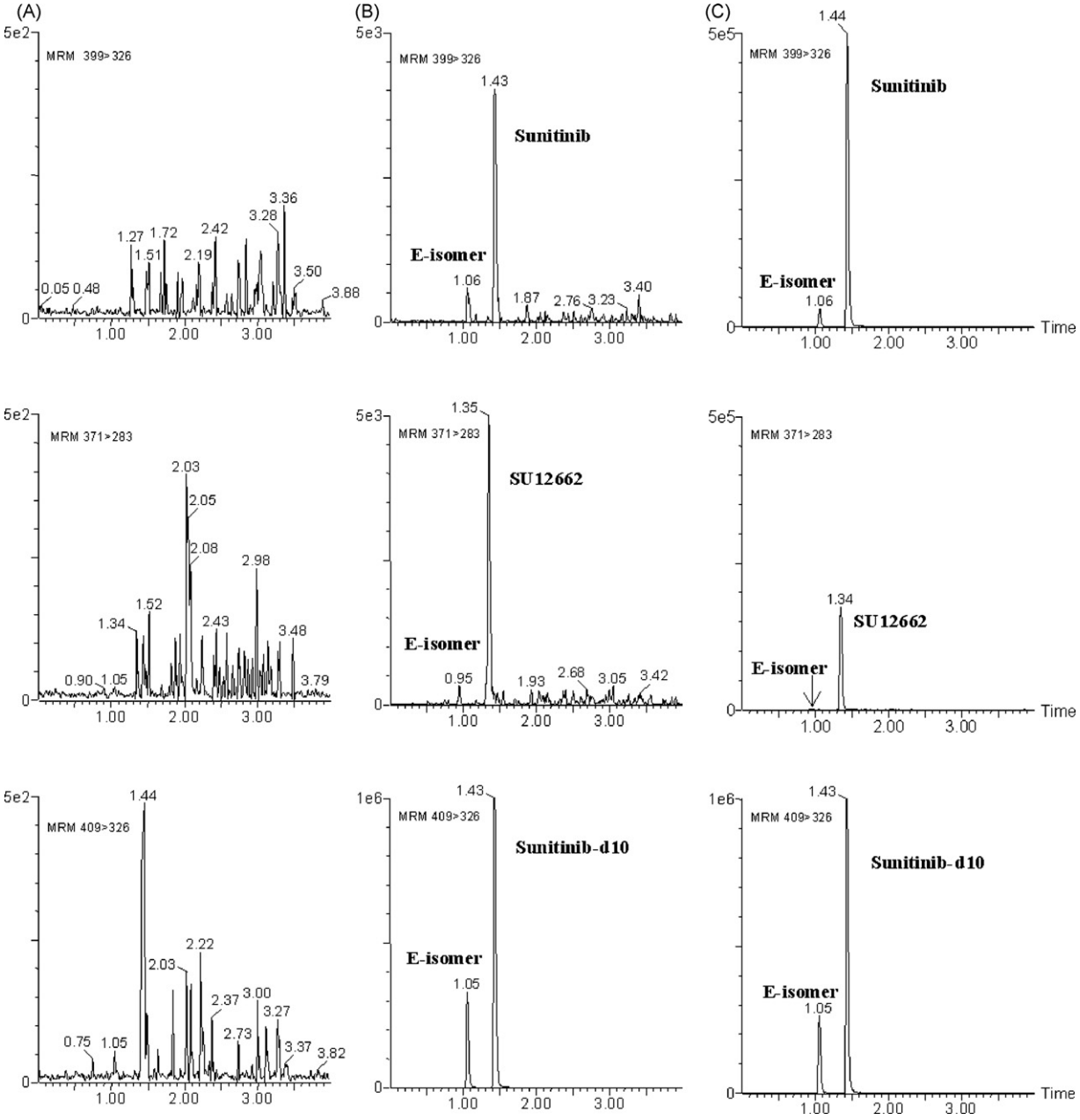

Fig. 2. Representative chromatograms of (A) a double blank processed plasma sample collected pre-dose prior to the first administration of 12.5 mg sunitinib, (B) a plasma sample spiked with 0.200 ng/ml sunitinib and SU12662 and (C) a plasma sample collected on day 15 containing 25.3 ng/ml sunitinib and 8.50 ng/ml SU12662.

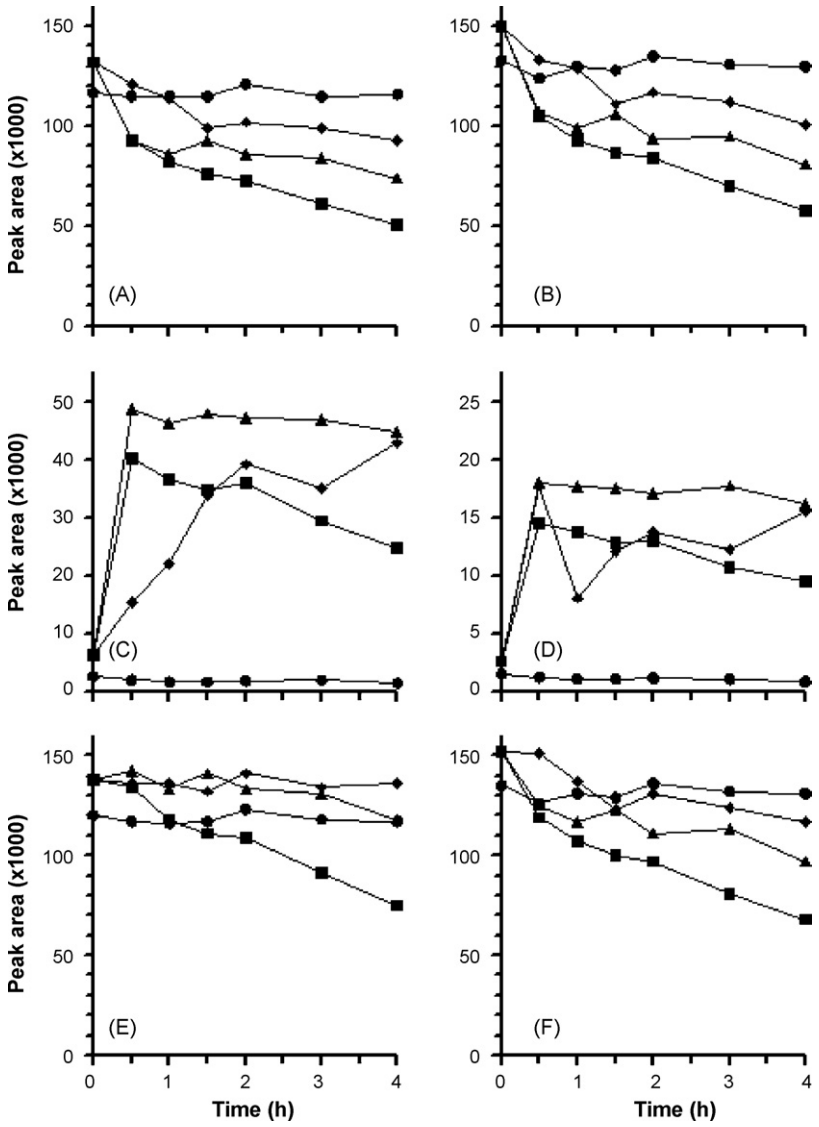

Fig. 3. Effect of light exposure on the conversion of the Z (*cis*)-isomer of sunitinib (A) and SU12262 (B), the formation of the E (*trans*)-isomer sunitinib (C) and SU12262 (D) and the stability of sunitinib (E) and SU12262 (F), expressed as the sum of the isomers following exposures to UV-light (squares), daylight (triangles) and sodium-light (lozenges). The circles represent the experiments in which the solutions were protected from light.

light (584 nm), a second set for 4 h to UV-light (254 nm) and the third set for 4 h to daylight (~350–700 nm), which were compared to the standard solution protected from light. As shown in Fig. 3A and B, no degradation of the Z (*cis*)-isomers of both sunitinib and SU12662 was observed when the solutions were protected from light. The largest decrease of the signals of the Z (*cis*)-isomers was seen when the solutions were exposed to UV-light (254 nm). Even in solutions exposed to sodium-light (589 nm) the signal of the Z (*cis*)-isomer decreased by approximately 30%, in contrast to the observations in a recent publication, were no evidence was found of photodegradation under yellow laboratory light (i.e. >500 nm) for the sunitinib related compound semaxanib [12].

The decreased signal of the Z (*cis*)-isomers was accompanied by an increased signal of the E (*trans*)-isomers (Fig. 3C and D). No significant formation of the E (*trans*)-isomers was seen during incubation of the solution protected from light.

As shown in Fig. 3E and F, no major decrease was seen in the sum of the responses of the E (*trans*)- and Z (*cis*)-isomers when protected from light or exposed to day- or sodium-light. This indicates that both isomers have equal mass spectral responses and no further degradation of the Z (*cis*)-isomer and the E (*trans*)-isomer occurred when the solutions were exposed to day- or sodium-light. In the solutions exposed to UV-light (254 nm), further degradation of the compounds occurred as shown by the decreased signal of the sum of the E (*trans*)- and Z (*cis*)-isomers.

As even the samples exposed to sodium-light showed photodegradation of the Z (*cis*)-isomer, samples should be processed in amber vials and under sodium-light to minimize the conversion of the Z (*cis*)-isomer to the E (*trans*)-isomer.

3.2. Assay performance

The method results were linear for both compounds ($r^2 \geq 0.9980$) in the concentration range of 0.200–50.0 ng/ml in human potassium EDTA plasma and none of the blank plasma samples showed potential interference for sunitinib, SU12662 or the labeled internal standard.

The LLQ was validated at 0.200 ng/ml. The measured concentrations of sunitinib for all 10 independently spiked plasma samples fell within the acceptable range of accuracy of 80–120%, with an average measured concentration of 0.208 ± 0.020 ng/ml. For

Table 1
Calculations of the between-run and within-run precisions and the average accuracy of the LLQ and QC samples.

Sample	Spiked (ng/ml)	GM (ng/ml)	WRP (%)	BRP (%)	ACC (%)	*n*
Sunitinib						
LLQ	0.200	0.181	11.7	#[a]	90.5	5
Low	0.600	0.559	8.89	6.52	93.2	5
Middle	25.0	25.1	3.09	1.75	100.4	5
High	40.0	40.2	2.47	1.14	100.5	5
Diluted	150	151	2.98	2.16	100.7	5
SU12662						
LLQ	0.200	0.190	8.03	1.15	95.0	5
Low	0.600	0.564	6.18	5.95	94.0	5
Middle	25.0	26.7	2.95	1.93	106.8	5
High	40.0	42.7	3.60	1.17	106.8	5
Diluted	150	158	3.53	#[a]	105.3	5

GM: grand mean; WRP: within-run precision; BRP: between-run precision; ACC: average accuracy; *n*: number of replicate observations on each analysis day.
[a] No additional variation observed by performing the assay in different runs.

Table 2
Extraction recovery (RE) and matrix effect (ME) in potassium EDTA plasma from six different lots spiked with sunitinib and SU12662 at a concentration of 40.0 ng/ml.

Plasma	Sunitinib		SU12662		Sunitinib-d10	
	RE (%)	ME (%)	RE (%)	ME (%)	RE (%)	ME (%)
Lot 1	108	83	110	83	100	78
Lot 2	112	81	112	84	116	74
Lot 3	92	88	96	89	100	82
Lot 4	95	99	92	111	99	94
Lot 5	96	86	99	86	108	81
Lot 6	102	80	102	82	108	75
Mean	101	86	102	89	105	81
S.D.	7.9	7.0	7.9	11	6.7	7.3

SU12662, also the back-calculated concentration of all 10 processed samples fell within the acceptable range of accuracy, with an average observed concentration of 0.199 ± 0.024 ng/ml. A representative chromatogram at the level of the LLQ is presented in Fig. 2B.

The within-run and between-run precisions and the accuracies at five tested concentrations in human potassium EDTA plasma, including at the level of the LLQ, are summarized in Table 1 and all fell within the accepted ranges as specified by the Food and Drug Administration (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf).

The extraction recovery (RE) and matrix effect (ME) were determined in potassium EDTA plasma from six different lots spiked with sunitinib and SU12662 at a concentration of 40.0 ng/ml. The mean measured extraction efficiencies for sunitinib, SU12662 and the internal standard (sunitinib-d10) were 101, 102 and 105%, respectively (Table 2). No major matrix effects were observed for sunitinib, SU12662 and the internal standard with values of 86, 89 and 81%, respectively (Table 2).

Sunitinib and its *n*-desethyl metabolite SU12662 were stable in potassium EDTA plasma at ambient temperature for at least 6 h, during three consecutive freeze–thaw cycles and as processed samples for a maximum of 22 h in the chilled (T = 10 °C) and light protected autosampler (Table 3).

None of the tested, potentially co-administered drugs interferes with the quantitation of sunitinib or SU12662 (data not shown). As the validated method will be used, amongst others, for the quantitation of sunitinib and SU12662 concentrations in patient samples enrolled in a clinical study, in which intravenous ifosfamide is given with or without oral sunitinib, samples collected during a ifosfamide infusion in the absence of sunitinib co-administration, were spiked with sunitinib. A remarkable ionization effect was seen on the response of sunitinib and the deuterated internal standard. In contrast, no ionization effect on SU12662 was seen. To further elucidate this phenomenon, potassium EDTA plasma containing 20 ng/ml sunitinib and SU12662 was spiked before extraction with ifosfamide at concentrations ranging from 0.05 to 50 μg/ml, which have been processed and compared to potassium EDTA plasma without ifosfamide. As shown in Fig. 4A, ifosfamide suppresses the ionization of sunitinib and its deuterated internal standard in a concentration dependent way, while no effect was observed for SU12662. It was confirmed that ifosfamide elutes at the same retention time as sunitinib (Fig. 4B). As the absolute ionization suppression on sunitinib and the internal standard are equal, the concentrations of sunitinib could be adequately quantitated, while the concentrations of SU12662 will be overestimated in the presence of high ifosfamide concentrations. Therefore, samples for the quantitation of SU12662, derived during the infusion of ifosfamide, needs to be diluted in blank plasma prior to processing in order to minimize the ionization suppression. As ifosfamide might be administered as 3 h infusions for 3 consecutive days up to continuous 3- or 5-day infusions [15,16], ifosfamide concentrations will differ markedly between schedules. Taking into consideration a maximum ionization effect of 15% as acceptable, ifosfamide concentrations, in diluted samples, should not exceed a concentration of 2 μg/ml (Fig. 4A).

Table 3
Stability of sunitinib and SU12662.[a].

	Sunitinb (ng/ml)		SU12662 (ng/ml)	
	(QC-Low)	(QC-High)	(QC-Low)	(QC-High)
In potassium EDTA plasma				
Directly processed	0.566 ± 0.044	40.1 ± 0.458	0.589 ± 0.040	42.7 ± 0.346
6 h at ambient temperature	0.592 ± 0.044 (105%)[b]	40.4 ± 1.66 (101%)[b]	0.594 ± 0.024 (101%)[b]	43.4 ± 1.25 (102%)[b]
3 freeze/thaw cycles	0.569 ± 0.034 (101%)[b]	40.6 ± 0.651 (101%)[b]	0.577 ± 0.033 (98.0%)[b]	43.6 ± 0.902 (102%)[b]
As processed samples in the autosampler				
Directly injected	0.565 ± 0.042	40.6 ± 1.04	0.639 ± 0.056	42.4 ± 1.45
After 6 h	0.565 ± 0.007 (100%)[c]	36.8 ± 1.62 (90.6%)[c]	0.553 ± 0.042 (86.5%)[c]	37.9 ± 2.89 (89.4%)[c]
After 22 h	0.483 ± 0.017 (85.5%)[c]	35.1 ± 0.361 (86.5%)[c]	0.505 ± 0.017 (79.0%)[c]	38.5 ± 0.321 (90.8%)[c]

[a] Data are presented as mean ± S.D. (n = 3).
[b] Mean concentration related to directly processed samples.
[c] Mean concentration related to directly injected samples.

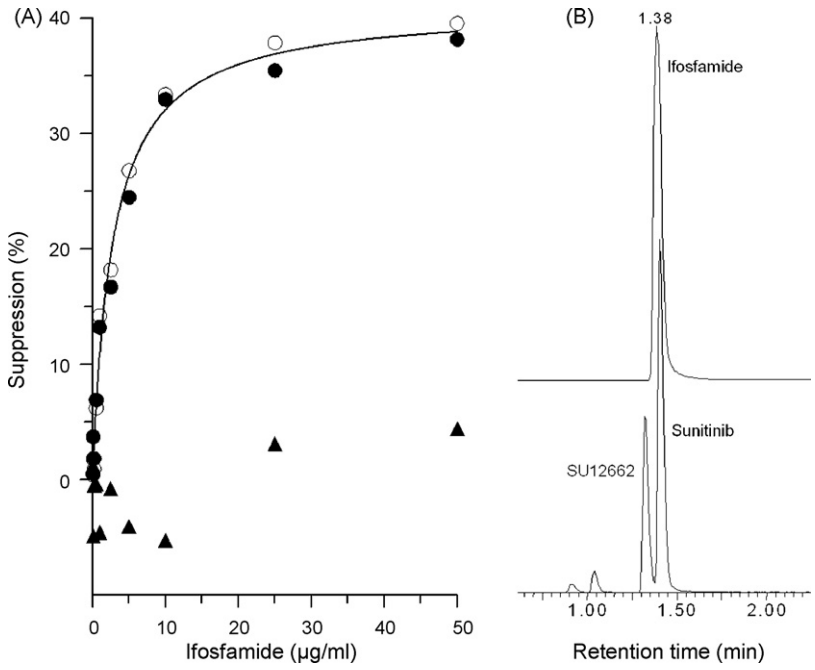

Fig. 4. Effect of ifosfamide on the ionization (A) of sunitinib (closed circles), SU12662 (triangles) and the deuterated internal standard sunitinib-d10 (open circles) and chromatogram of ifosfamide, sunitinib and SU12662 (B).

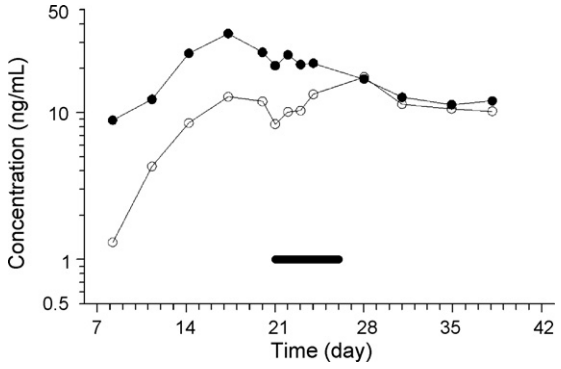

Fig. 5. Plasma concentration–time profile of sunitinib (closed symbols) and its metabolite SU12662 (open symbols) after an oral dose of 12.5 mg/daily. The bar represents the duration of the 5-day continuous intravenous ifosfamide infusion at a dose of 1200 mg/m^2/day.

3.3. Clinical application

The described analytical method was applied in our institute to a pharmacokinetic study of intravenous ifosfamide in combination with oral sunitinib. A representative plasma concentration–time profile of sunitinib and its metabolite SU12662 after an oral dose of 12.5 mg/daily is shown in Fig. 5. Representative chromatograms of this patient are shown in Fig. 2A and C.

4. Conclusion

A selective, sensitive, accurate, and precise method has been validated for sunitinib and its *n*-desethyl metabolite SU12662 in human potassium EDTA plasma, which meets the current requirements of bioanalytical method validation (www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf). It is of note that sunitinib and SU12662 are both very light sensitive and samples should be processed in amber vials, protected from any light source as much as possible. Furthermore, the observed ionization suppression of ifosfamide on sunitinib underlines the importance of a decent investigation of the potential interference of co-administered drugs on the validated analytes in any bioanalytical method.

References

[1] S. Faivre, C. Delbaldo, K. Vera, C. Robert, S. Lozahic, N. Lassau, C. Bello, S. Deprimo, N. Brega, G. Massimini, J.P. Armand, P. Scigalla, E. Raymond, Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer, J. Clin. Oncol. 24 (2006) 25–35.
[2] G.D. Demetri, A.T. van Oosterom, C.R. Garrett, M.E. Blackstein, M.H. Shah, J. Verweij, G. McArthur, I.R. Judson, M.C. Heinrich, J.A. Morgan, J. Desai, C.D. Fletcher, S. George, C.L. Bello, X. Huang, C.M. Baum, P.G. Casali, Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial, Lancet 368 (2006) 1329–1338.
[3] R.J. Motzer, T.E. Hutson, P. Tomczak, M.D. Michaelson, R.M. Bukowski, O. Rixe, S. Oudard, S. Negrier, C. Szczylik, S.T. Kim, I. Chen, P.W. Bycott, C.M. Baum, R.A. Figlin, Sunitinib versus interferon alfa in metastatic renal-cell carcinoma, N. Engl. J. Med. 356 (2007) 115–124.
[4] G.D. Demetri, Y. Wang, E. Wehrle, A. Racine, Z. Nikolova, C.D. Blanke, H. Joensuu, M. von Mehren, Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors, J. Clin. Oncol. 27 (2009) 3141–3147.
[5] B.E. Houk, C.L. Bello, M.D. Michaelson, R.M. Bukowski, B.G. Redman, G.R. Hudes, G. Wilding, R.J. Motzer, Exposure-response of sunitinib in metastatic renal cell carcinoma (mRCC): a population pharmacokinetic/pharmacodynamic (PKPD) approach, J. Clin. Oncol., 2007 ASCO Annual Meeting Proceedings Part I, vol. 25, no. 18S, abstract 5027 (June 20 Suppl.), 2007.
[6] N.P. van Erp, K. Eechoute, A.A. van der Veldt, J.B. Haanen, A.K. Reyners, R.H. Mathijssen, E. Boven, T. van der Straaten, R.F. Baak-Pablo, J.A. Wessels, H.J. Guchelaar, H. Gelderblom, Pharmacogenetic pathway analysis for determination of sunitinib-induced toxicity, J. Clin. Oncol. 27 (2009) 4406–4412.
[7] C.L. Bello, L. Sherman, J. Zhou, L. Verkh, J. Smeraglia, J. Mount, K.J. Klamerus, Effect of food on the pharmacokinetics of sunitinib malate (SU11248), a multitargeted receptor tyrosine kinase inhibitor: results from a phase I study in healthy subjects, Anticancer Drugs 17 (2006) 353–358.
[8] C.D Britten, F. Kabbinavar, J.R. Hecht, C.L. Bello, J. Li, C. Baum, D. Slamon, A phase I and pharmacokinetic study of sunitinib administered daily for 2 weeks, followed by a 1-week off period, Cancer Chemother. Pharmacol. 61 (2008) 515–524.
[9] W. Fiedler, H. Serve, H. Dohner, M. Schwittay, O.G. Ottmann, A.M. O'Farrell, C.L. Bello, R. Allred, W.C. Manning, J.M. Cherrington, S.G. Louie, W. Hong, N.M. Brega, G. Massimini, P. Scigalla, W.E. Berdel, D.K. Hossfeld, A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease, Blood 105 (2005) 986–993.
[10] J.O. Haznedar, S. Patyna, C.L. Bello, G.W. Peng, W. Speed, X. Yu, Q. Zhang, J. Sukbuntherng, D.J. Sweeny, L. Antonian, E.Y. Wu, Single- and multiple-dose

disposition kinetics of sunitinib malate, a multitargeted receptor tyrosine kinase inhibitor: comparative plasma kinetics in non-clinical species, Cancer Chemother. Pharmacol. 64 (2009) 691–706.
[11] P. Minkin, M. Zhao, Z. Chen, J. Ouwerkerk, H. Gelderblom, S.D. Baker, Quantification of sunitinib in human plasma by high-performance liquid chromatography–tandem mass spectrometry, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 874 (2008) 84–88.
[12] Y. Zhao, J. Sukbuntherng, L. Antonian, Simultaneous determination of Z-SU5416 and its interconvertible geometric E-isomer in rat plasma by LC/MS/MS, J. Pharm. Biomed. Anal. 35 (2004) 513–522.
[13] P. de Bruijn, I.M. Moghaddam-Helmantel, M.J. de Jonge, T. Meyer, M.H. Lam, J. Verweij, E.A. Wiemer, W.J. Loos, Validated bioanalytical method for the quantification of RGB-286638, a novel multi-targeted protein kinase inhibitor, in human plasma and urine by liquid chromatography/tandem triple-quadrupole mass spectrometry, J. Pharm. Biomed. Anal. 50 (2009) 977–982.
[14] H. Rosing, W.Y. Man, E. Doyle, A. Bult, J.H. Beijnen, Bioanalytical liquid chromatographic method validation. A review of current practices and procedures, J. Liq. Chromatogr. Rel. Technol. 23 (2000) 329–354.
[15] E.G. Brain, K. Rezai, S. Weill, M.F. Gauzan, J. Santoni, B. Besse, A. Goupil, F. Turpin, S. Urien, F. Lokiec, Variations in schedules of ifosfamide administration: a better understanding of its implications on pharmacokinetics through a randomized cross-over study, Cancer Chemother. Pharmacol. 60 (2007) 375–381.
[16] R. Coriat, O. Mir, S. Camps, S. Ropert, B. Billemont, M. Leconte, F. Larousserie, P. Anract, J. Alexandre, F. Goldwasser, Ambulatory administration of 5-day infusion ifosfamide + mesna: a pilot study in sarcoma patients, Cancer Chemother. Pharmacol. (2009) (Epub ahead of print).