Quantification of 11 Therapeutic Kinase Inhibitors in Human Plasma for Therapeutic Drug Monitoring Using Liquid Chromatography Coupled With Tandem Mass Spectrometry

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Background: A liquid chromatography/tandem mass spectrometry assay was developed to facilitate therapeutic drug monitoring (TDM) for 10 anticancer compounds (dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, sorafenib, sunitinib, and vemurafenib) and the active metabolite, N-desethyl-sunitinib.

Methods: The TDM assay is based on reversed-phase chromatography coupled with tandem mass spectrometry in the positive ion mode using multiple reaction monitoring for analyte quantification. Stable isotopically labeled compounds were used as internal standards. The sample pretreatment consisted of protein precipitation with acetonitrile using a small plasma volume of 50 μL. The validation procedures were based on the guidelines on bioanalytical methods issued by the US Food and Drug Administration and were modified to fit the requirements of the clinical TDM environment.

Results: The method was validated over a linear range of 5.00–100 ng/mL for dasatinib, sunitinib, and N-desethyl-sunitinib; 50.0–1000 ng/mL for gefitinib and lapatinib; 125–2500 ng/mL for erlotinib, imatinib, and nilotinib; and 500–10,000 ng/mL for pazopanib, sorafenib, and vemurafenib. The results of the validation study demonstrated good intra-assay and interassay accuracy (bias <6.0%) and precision (12.2%) for all analytes.

Conclusions: This newly validated method met the criteria for TDM and has successfully been applied to routine TDM service for tyrosine kinase inhibitors.

Key Words: LC-MS/MS, therapeutic drug monitoring, tyrosine kinase inhibitors, validation, clinical application

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INTRODUCTION

Therapeutic drug monitoring (TDM) of anticancer drugs is becoming an important tool in the targeted treatment of patients with cancer, and tyrosine kinase inhibitors (TKIs) are among the class of targeted drug therapies. Almost all drugs in this rapidly growing group are subject to high pharmacokinetic variability. TDM has been increasingly useful in managing the considerable interindividual variability that is observed in the pharmacokinetics of these drugs.[1–7] Variations in absorption, distribution, metabolism, and excretion in and between individuals may be because of food intake, concomitant medication, underlying disease, age, genetics, and other factors.[8]Consequently, drug levels vary considerably and this may lead to insufficient efficacy or substantial toxicity. TDM aims to individualize drug dosing by focusing on balancing the therapeutic efficacy and the avoidance of drug toxicity.[9,10] This is achieved by quantifying drug concentrations in patient blood plasma or serum and by comparing the results with predetermined guidelines and target levels.

Guidelines for the TDM of TKIs are sparse in the literature; however, previous literature reviews by various authors have provided information on pharmacokinetic targets that may be used in the TDM practice.[11–13] A rapidly performed, robust, and adequately ranged TDM assay for a large variety of frequently used TKIs is not currently available in routine clinical practice. Therefore, to meet this need, we developed and validated a bioanalytical assay devoted to the TDM of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, sorafenib, sunitinib, N-desethyl-sunitinib, and vemurafenib. The validation procedures were designed according to the US Food and Drug Administration (FDA) Guidelines,[14,15] with the following modifications. (1) Four nonzero calibrators were used in the calibration curve instead of 6 to 8. (2) The quality control low concentration sample was replaced with a QC lower limit of quantification (LLOQ) sample for the determination of the inaccuracy and imprecision. This allowed for the development of a method that is routinely useable in the time-limited clinical practice while still offering a bioanalytical validation approach. This article describes the validation and application of the

quantitative analysis of 10 TKIs and a metabolite in human plasma for TDM purposes. After protein precipitation, the samples were analyzed using ultra-performance liquid chromatography (UPLC) system equipped with a high-performance liquid chromatography (HPLC) column with tandem mass spectrometry detection.

This method was based on an assay that was developed by our laboratory for the quantitative analysis of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib, and sunitinib in human plasma,[16] and was adapted to quantify additional analytes. Additional changes were made to allow for a shorter analysis time as well as its use in a TDM setting. The validated ranges of the method were chosen to include the mean observed C_{trough} levels of the analytes, which Yu et al[11] found to be higher than the suggested $C_{through}$ levels for most TKIs. This assured a proper range for both measurement and clinical interpretation in the future. Subsequently, the applicability of the method in analyzing clinical samples was demonstrated.

MATERIALS AND METHODS

Chemicals

The analytes and internal standards were purchased from the following indicated sources. Dasatinib, erlotinib hydrochloride, gefitinib, imatinib mesylate, lapatinib ditosylate, nilotinib, sunitinib maleate, sorafenib tosylate, and vemurafenib were from Sequoia Research Products (Pangbourne, United Kingdom). Erlotinib-$^{13}C_6$ hydrochloride, gefitinib-$^{2}H_3$, imatinib-^{13}C and $^{2}H_3$, lapatinib-^{13}C and $^{2}H_7$, pazopanib-^{13}C and $^{2}H_3$ hydrochloride, sunitinib-$^{2}H_{10}$, sorafenib-^{13}C and $^{2}H_3$, and vemurafenib-$^{13}C_6$ were from AlsaChim (Illkirch, France). Pazopanib hydrochloride was from GlaxoSmithKline (London, United Kingdom), whereas the N-desethyl sunitinib, dasatinib-$^{2}H_8$, nilotinib-$^{2}H_3$, and N-desethyl-sunitinib-$^{2}H_5$ were from Toronto Research Chemicals (Toronto, ON, Canada). The chemical formulas and masses of the analytes are presented in Table 1. Acetonitrile, isopropanol, and methanol were purchased from Biosolve Ltd (Amsterdam, the Netherlands). Ammonium, formic acid, and LiChrosol water for HPLC were obtained from Merck (Darmstadt, Germany). Small volumes of control drug-free human plasma obtained from the Slotervaart Hospital (Amsterdam, the Netherlands) were pooled and used for the validation.

TABLE 1. Molecular Formulas and Masses of Analytes

Generic Drug Name	Molecular Formula	Molecular Mass, Da
Dasatinib	$C_{22}H_{26}Cl_1N_7O_2S_1$	488.0
Erlotinib	$C_{22}H_{23}N_3O_4$	393.4
Gefitinib	$C_{22}H_{24}ClFN_4O_3$	446.9
Imatinib	$C_{29}H_{31}N_7O$	493.6
Lapatinib	$C_{29}H_{26}ClFN_4O_4S$	581.1
N-desethyl-sunitinib	$C_{20}H_{23}FN_4O_2$	370.4
Nilotinib	$C_{29}H_{22}F_3N_7O$	529.5
Pazopanib	$C_{21}H_{23}N_7O_2S$	437.5
Sorafenib	$C_{21}H_{16}N_4ClF_3O_3$	465.1
Sunitinib	$C_{22}H_{27}FN_4O_2$	398.5
Vemurafenib	$C_{23}H_{18}ClF_2N_3O_3S$	489.9

Chromatographic Equipment and Conditions

A Waters Acquity I class UPLC system, consisting of a binary pump, column oven, on-line degasser, and an autosampler (Waters, Milford, MA) was used. The mobile phase A consisted of a 10 mmol/L mixture of ammonium hydroxide (NH_4OH) in water and mobile phase B was 1 mmol/L NH_4OH in methanol. The UPLC system was equipped with a Gemini C18 HPLC column (50 × 2.0 mm I.D., 5.0-μm; Phenomenex, Torrance, CA) and the flow rate was set to 0.25 mL/min. The analytical column was protected in-line by a Gemini C18 column (4 × 2.0 mm I.D., 5.0-μm; Phenomenex). The column was kept at 40°C, the autosampler was set at 8°C, and the injection volume was 1 μL. The autosampler needle was rinsed with a mixture of acetonitrile/methanol/isopropanol/water (1/1/1/1, vol/vol/vol/vol) containing 0.1% formic acid. During the first 0.5 minutes, the eluate was directed to the waste container using a divert valve to prevent contamination of the mass spectrometer. A rapid gradient program was used to achieve the separation. The initial conditions were 55% mobile phase B, which was maintained for 0.5 minutes. In the following 1.5 minutes, the mobile phase was increased to 80% B. After 1.5 minutes and at 80% B, the initial conditions were restored with a total run time of 5.5 minutes.

MS Equipment and Conditions

An API 5500 triple quadrupole mass spectrometer equipped with a turbo ion spray source (Sciex, Foster City, CA) operating in the positive ion mode was used as the detector. For the quantification, multiple-reaction monitoring chromatograms were acquired and processed using the Analyst software version 1.6 (Sciex). The quadrupoles were operated at unit resolution (0.7 Da). The operating parameters of the system and mass transitions are listed in Table 2.

Preparation of Calibrators, Quality Control Samples, and Internal Standard Solutions

Two separate stock solutions of all analytes (1 or 2 mg/mL) and internal standards (0.5 or 1 mg/mL) were prepared. Exactly 1 mg was weighed and dissolved in 1 mL of methanol. For the analytes, 1 stock solution was used to prepare the calibrators and the other stock for the preparation of the QC standards. The preparation of the 2 stock solutions for each compound was checked and in all cases, deviations were $< \pm 5\%$. The stock solutions were further diluted with methanol to obtain separate working solutions each containing all the analytes at a 20-fold concentration of the corresponding plasma samples.

The calibrators were freshly prepared for every validation run by spiking a volume of 50 μL working solution to 1.0 mL of control human plasma. The QC samples

TABLE 2. Tandem Mass Spectrometry (MS/MS) Operating Parameters

Parameter	Setting
Run duration	5.50 min
Ion spray voltage	4500 V
Collision gas	9 psi
Curtain gas	20 psi
Turbo gas	40 psi
Internal Standard (IS) temperature	500°C

Specific Parameters Analyte	Parent Mass, m/z	Product Mass, m/z	Collision Energy, V	Declustering Potential, V	Collision Exit Potential, V
Dasatinib	488	401	45	236	42
Dasatinib-$^{2}H_8$	496	406			
Erlotinib	394	278	45	236	34
Erlotinib-$^{13}C_6$	400	284			
Gefitinib	447	128	13	131	20
Gefitinib-$^{2}H_3$	455	136			
Imatinib	494	394	43	46	42
Imatinib-$^{13}C,^{2}H_3$	498	394			
Lapatinib	581	365	57	111	36
Lapatinib-$^{13}C,^{2}H_7$	589	365			
N-desethyl-sunitinib	371	283	27	126	40
N-desethyl-sunitinib-$^{2}H_5$	376	283			
Nilotinib	530	289	47	36	10
Nilotinib-$^{2}H_3$	533	289			
Pazopanib	438	357	11	136	14
Pazopanib-$^{13}C,^{2}H_3$	442	361			
Sorafenib	465	252	49	176	28
Sorafenib-$^{13}C,^{2}H_3$	469	256			
Sunitinib	399	326	31	106	44
Sunitinib-$^{2}H_{10}$	409	326			
Vemurafenib	490	383	45	196	38
Vemurafenib-$^{13}C_6$	496	389			

were prepared in batches and stored at −20°C until analysis. A mixture of the internal standard stock solutions was prepared and diluted with methanol to obtain a working solution that was used for sample preparation (Table 3 lists the concentrations).

Sample Preparation

Acetonitrile was used for protein precipitation in the sample pretreatment. To 50 μL each of plasma and the internal standard working solution, 1000 μL acetonitrile was added. The mixture was vortexed for 10 seconds, and then centrifuged for 10 minutes at 11,300*g* at 20–25°C. A 500-μL aliquot of the mixture was transferred to an autosampler vial and 500 μL 100 mmol/L NH_4OH in water was added. The vials were capped, vortexed for 10 seconds, and then stored at 2–8°C until analysis.

Validation Procedures

The assay was validated according to the FDA Guidelines, and adjustments were made for the process to fit TDM purposes.[14,15]

Regression Models

Four nonzero calibrators were prepared in duplicate for each run and analyzed in 3 separate independent runs. The linearity was evaluated using back-calculated concentrations of the calibrators. For all analytes, the reciprocal of the squared concentration ($1/x^2$) was used as the weighting factor. The deviations from the mean calculated concentrations over 3 runs were expected to be within ±15% of the nominal concentrations. At the LLOQ, a deviation of ±20% was permitted and the response of the analyte was expected to be at least 5 times higher than that of the blank sample.

Inaccuracy and Imprecision

Intra-assay and interassay inaccuracies and imprecisions of the method were determined by quantifying 5 replicates of each of the QC samples at the LLOQ, midrange level (MID), and upper limit of quantification (ULOQ) concentration level in 3 separate runs. The concentration of each QC sample was calculated using the calibrators that were analyzed in duplicate in the same run. The difference between the nominal and the measured concentration were used to calculate the inaccuracies. The

TABLE 3. Concentrations of Stock and Calibrators

Analyte Solution	DSN	ELN	GFN	IMN	LPN	mSNN	NLN	PZN	SRN	SNN	VMN
Analyte stock, mg/mL	1	2	2	2	2	1	2	2	2	1	2
IS stock, mg/mL	1	0.5	1	1	0.5	0.5	0.5	1	1	0.5	1
CAL4/ULOQ, ng/mL	100	2500	1000	2500	1000	100	2500	10,000	10,000	100	10,000
CAL3/MID, ng/mL	50	1250	500	1250	500	50	1250	5000	5000	50	5000
CAL2, ng/mL	10	250	100	250	100	10	250	1000	1000	10	1000
CAL1/LLOQ, ng/mL	5	125	50	125	50	5	125	500	500	5	500
IS working, ng/mL	50	1250	500	1250	500	50	1250	5000	5000	50	5000

DSN, dasatinib; ELN, erlotinib; GFN, gefitinib; IMA, imatinib; IS, internal standard; LPN, lapatinib; mSNN, N-desethyl-sunitinib; NLN, nilotinib; PZN, pazopanib; SNN, sunitinib; SRN, sorafenib; VMN, vemurafenib.

inaccuracy (bias) was expected to be ≤15% for MID and ULOQ, and ≤20% for the LLOQ. The imprecision (% coefficient of variation) was expected to be ≤15% for the MID and ULOQ level and ≤20% at the LLOQ level. The ability to dilute samples with an analyte concentration that was originally above the ULOQ was demonstrated for pazopanib and vemurafenib by analyzing QC samples containing 10 times the concentration of the high QC sample. These samples were prepared as 5-fold concentrations and were analyzed after a 10-fold dilution with the control human plasma.

TABLE 4. Assay Performance Data

Analyte	Nominal Concentration, ng/mL	Intra-assay Bias, %	Intra-assay CV,* %	Inter-assay Bias, %	Inter-assay CV, %
Dasatinib	4.97	4.1	15.9	3.0	11.5
	49.7	10.2	7.2	−0.5	9.5
	99.4	6.8	7.7	1.6	7.9
Erlotinib	128	−4.7	2.7	−1.7	3.4
	1280	2.5	1.1	2.1	1.1
	2550	−3.5	1.8	−1.6	2.0
Gefitinib	48.6	−12.1	11.5	−6.0	10.6
	486	10.6	5.7	5.5	7.0
	972	−5.6	5.2	−0.9	5.0
Imatinib	130	6.5	4.3	3.2	3.6
	1300	1.4	2.3	0.1	1.8
	2600	−2.3	2.5	−1.7	2.1
Lapatinib	51.0	−8.7	5.2	−3.9	5.6
	510	4.2	2.8	1.0	3.4
	1020	2.2	1.6	0.1	2.0
N-desethyl-sunitinib	5.11	−14.1	12.2	−5.1	12.2
	51.1	−7.4	12.0	−1.7	10.3
	102	−3.1	8.8	−1.3	6.3
Nilotinib	125.0	−4.8	4.6	−1.2	4.9
	1250	2.9	1.5	2.3	1.2
	2500	−4.8	2.3	−3.7	2.0
Pazopanib	502.0	8.8	9.1	4.4	8.7
	5020	10.1	4.6	4.8	5.0
	10,000	−7.7	4.2	−0.9	5.9
Sunitinib	4.87	−5.2	14.9	−1.0	9.6
	48.7	3.9	6.5	1.9	4.8
	97.5	4.4	5.7	−0.8	5.6
Sorafenib	497	−3.8	2.2	−1.3	2.6
	4970	4.1	1.0	3.0	1.1
	9940	−6.7	1.3	−5.6	1.4
Vemurafenib	480	2.4	2.4	0.5	3.2
	4800	2.5	2.3	1.5	1.9
	9600	−2.0	3.5	−0.5	2.4

*CV, coefficient of variation (n = 5 per run, n = 15 in total).

Carryover

The carryover was determined by injecting 2 processed control human plasma samples after a ULOQ sample. The peak areas in the blank processed sample were expected to be ≤20% of the peak area of the LLOQ sample.

Specificity and Selectivity

Six individual batches of control human plasma were used to assess the specificity and selectivity of the method. To determine whether any endogenous constituents interfered with the assay, a double blank and a sample spiked at the LLOQ of each batch were processed. The samples were then analyzed using the procedures described above. The areas of the peaks coeluting with the analytes were expected to be ≤20% of the peak area of the LLOQ sample in each of the 6 batches. The areas of peaks in the double blanks coeluting with the internal standards were expected to be ≤5% of the peak area of the mean internal standard response. For the LLOQ, sample inaccuracies were expected to be within ±20% of the nominal concentration in 4 of the 6 samples.

Stability

The stability of the analytes was investigated in the stock solutions at an ambient temperature (2 hours) and at −20°C (1 month). The stability was also tested in human plasma for 48 hours at 20–25°C, for 1 month at −20°C, and after 3 freeze–thaw cycles (−20°C to 20–25°C) with a minimum interval of 24 hours at 2 concentrations. The processed sample stability was investigated after 8 days of storage at 2–8°C at 2 concentrations. The stability samples were quantified using freshly prepared calibrators. Reinjection reproducibility was determined in the processed sample after 24 hours at approximately 2–8°C at 3 concentrations. All stability experiments were performed in triplicate. The analytes were considered stable in the stock solutions when 95%–105% of the original

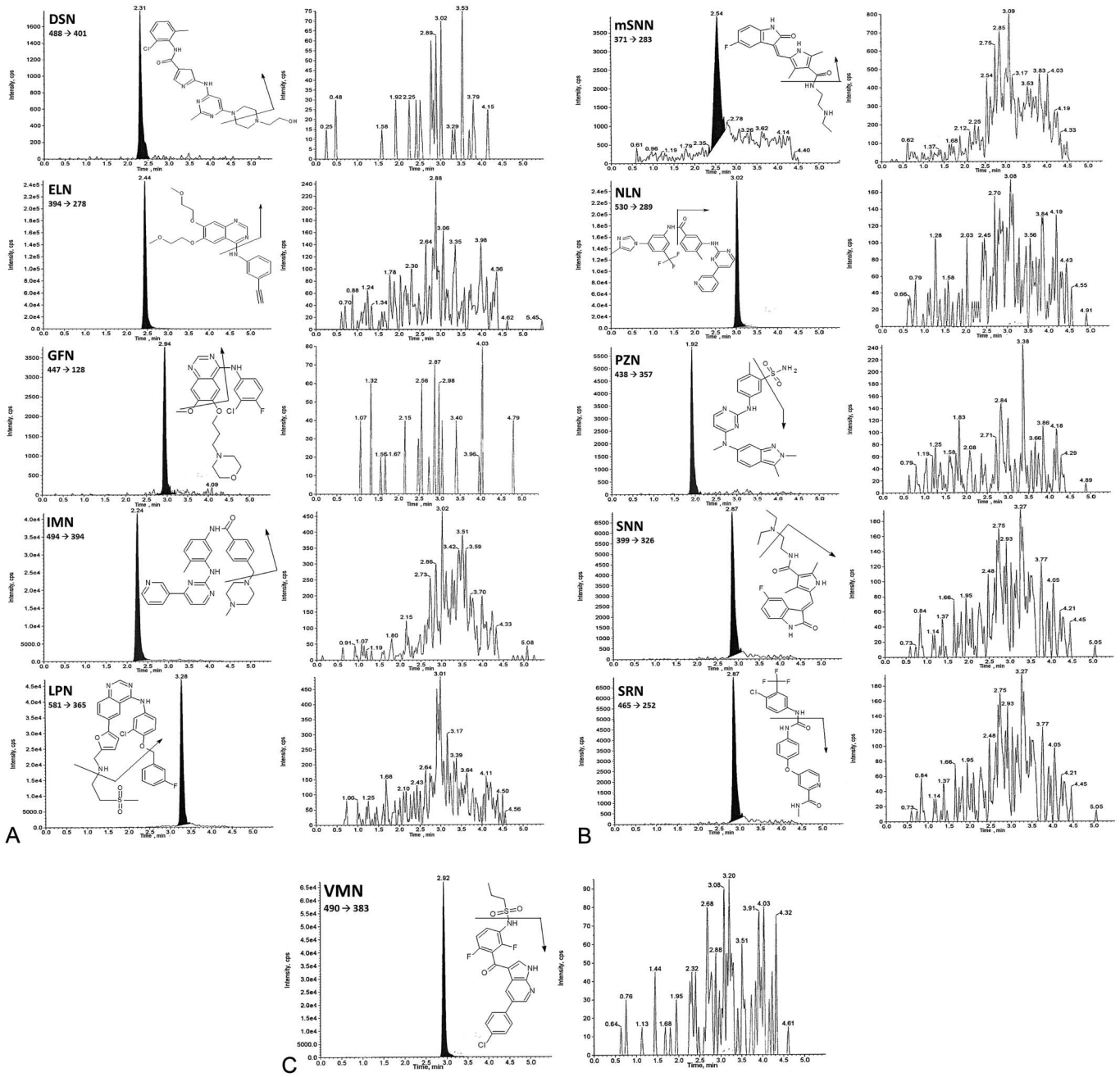

FIGURE 1. A, Chromatograms of spiked plasma samples at lower limit of quantification [LLOQ, bracketed concentrations (ng/mL), left] with proposed fragmentation pathways and blank samples (right) TKIs: DSN, dasatinib (5.00); ELN, erlotinib (125); GFN, gefitinib (50.0); IMN, imatinib (125); LPN, lapatinib (50.0). B, Chromatograms of spiked plasma samples at lower limit of quantification [LLOQ, bracketed concentrations (ng/mL), left] with proposed fragmentation pathways and blank samples (right) TKIs: mSNN, N-desethyl-sunitinib (5.00); NLN, nilotinib (125); PZN, pazopanib (500); SNN, sunitinib (5.00); SRN, sorafenib (500). C, Chromatogram of spiked plasma sample at lower limit of quantification [LLOQ, bracketed concentration (ng/mL), left] with proposed fragmentation pathway and blank sample (right) TKIs: VMN, vemurafenib (500).

concentration was detected. The analytes were considered stable in the plasma or processed sample when 85%–115% of the initial concentration was recovered. The stability of the labeled internal standards was assumed to be equal to that of the corresponding unlabeled analytes. The isotopic purity of the internal standards was investigated during each analytical run by spiking control human plasma with the internal standard working solution. The peak area in the unlabeled isotope analyte window was expected to be <20% of the peak area of the analytes at their LLOQ level.

Applicability of the Method for TDM

The applicability of the assay was demonstrated by analyzing the steady-state plasma concentrations of patients receiving the regular prescribed doses for the registered indications of the TKIs. Additionally, steady-state plasma concentrations of N-desethyl-sunitinib were measured in the plasma of patients receiving sunitinib.

RESULTS

Regression Models

All calibration curves were constructed using a weighting factor of $1/x^2$ and fitted linearly. The assay was linear for the validated concentration ranges of 5.00–100 ng/mL for dasatinib, sunitinib, and N-desethyl-sunitinib; 50.0–1000 ng/mL for gefitinib and lapatinib; 125–2500 ng/mL for erlotinib, imatinib, and nilotinib; and 500–10,000 ng/mL for pazopanib, sorafenib, and vemurafenib. The correlation coefficients (r^2) were at least 0.995 and the accuracies were within 85%–115% in all cases. At all concentration levels, the coefficient of variations were ≤15% for the analytes.

Inaccuracy and Imprecision

The assay performance (intra-assay and interassay inaccuracy and imprecision) of all analyzed compounds is summarized in Table 4. The intra-assay and interassay biases were ≤15% for the MID and ULOQ levels and ≤20% for the LLOQ levels. The imprecisions were ≤15% for the MID and ULOQ levels and ≤20% for the LLOQ levels.

Carryover

The areas of the peaks in the first blank processed sample were ≤20% of the peak area of the LLOQ sample for all analytes except for lapatinib, which required an extra blank sample.

Specificity and Selectivity

The multiple-reaction monitoring chromatograms of 6 batches of the control human plasma contained no coeluting peaks that were >20% of the area at the LLOQ level for all analytes and no coeluting peaks >5% of the area of all internal standards. Figure 1 shows representative chromatograms of both LLOQ and blank samples. The influence of different control human plasma batches on the accuracy and precision at the LLOQ level was also investigated. The inaccuracies of the analytes at the LLOQ level were within ±20% of the nominal concentrations for all 6 batches of the control human plasma.

Stability

The stability of the analytes in various solutions and concentrations was investigated. The stock solutions were stable for at least 2 hours at 20–25°C. Plasma samples were stable for at least up to 48 hours at 20–25°C and for 1 month at −20°C. The calibrators and QC samples were aliquoted, stored at −20°C, and thawed directly before processing to minimize the number of freeze–thaw cycles. The stability in plasma was guaranteed for at least 3 freeze (−20°C)–thaw cycles. The processed samples were stable for at least 8 days at 2–8°C. The reinjection reproducibility after 24 hours at approximately 2–8°C was ensured. After storing the working solution for 6 months at −20°C, the peak areas of the analytes in the processed control human plasma sample spiked with the internal standard working solution were ≤20% of the peak areas of the analytes in an LLOQ sample.

TABLE 5. Analyte Concentrations in Plasma Collected From Patients Receiving Standard Therapy

Drug	Mean Plasma Concentration, ng/mL	Min.-Max. Concentrations, ng/mL
Dasatinib	22.0	8.76–45.6
Erlotinib	993	259–1770
Gefitinib	305	89–761
Imatinib	1522	581–2170
Lapatinib	221	65.9–828
N-desethyl-sunitinib	18.3	7.94–32.3
Nilotinib	1615	603–2280
Pazopanib	40,800*	13,500*–82,900*
Sunitinib	49.1	5.51–94.2
Sorafenib	2998	1120–5530
Vemurafenib	42,911*	12,300*–90,000*

*Plasma samples were diluted 10 times before sample preparation, 10 patients/analyte.

Method Applicability for TDM

The results of the analyses of 10 plasma concentrations per inhibitor and N-desethyl sunitinib are presently reported here. The mean measured concentrations are shown in Table 5 along with the lowest and highest measured plasma concentrations. Figure 2 shows typical chromatograms of a patient sample for each analyte.

DISCUSSION

A previously developed assay for the analysis of multiple TKIs was improvised with regard to the number of analytes that can be analyzed per run, the overall time of analysis, and its applicability to TDM routines.[6] The previous assay was developed for the analysis of 8 compounds, and pazopanib, N-desethyl-sunitinib, and vemurafenib were added in the improved version. The sample preparation and the concentration range were altered along with these changes. Additionally, the chromatographic conditions were adjusted and, thereby, the total analysis time could be reduced from 10 to 5.5 minutes. The gradient profile was modified from a 3-to a 2-step profile while maintaining a flow of 0.25 mL/min. Typical chromatograms of samples at the LLOQ are depicted in Figure 1. To develop a method more suitable for an efficient daily TDM assay, the bioanalytical set-up was changed. The previous assay had a larger calibration range (ULOQ = 400 × LLOQ), which was scaled down in the new assay (ULOQ = 20 × LLOQ). The number of calibrators was reduced to 4, which provided a full calibration curve coverage with a shortened preparation time. The QC was carried out at

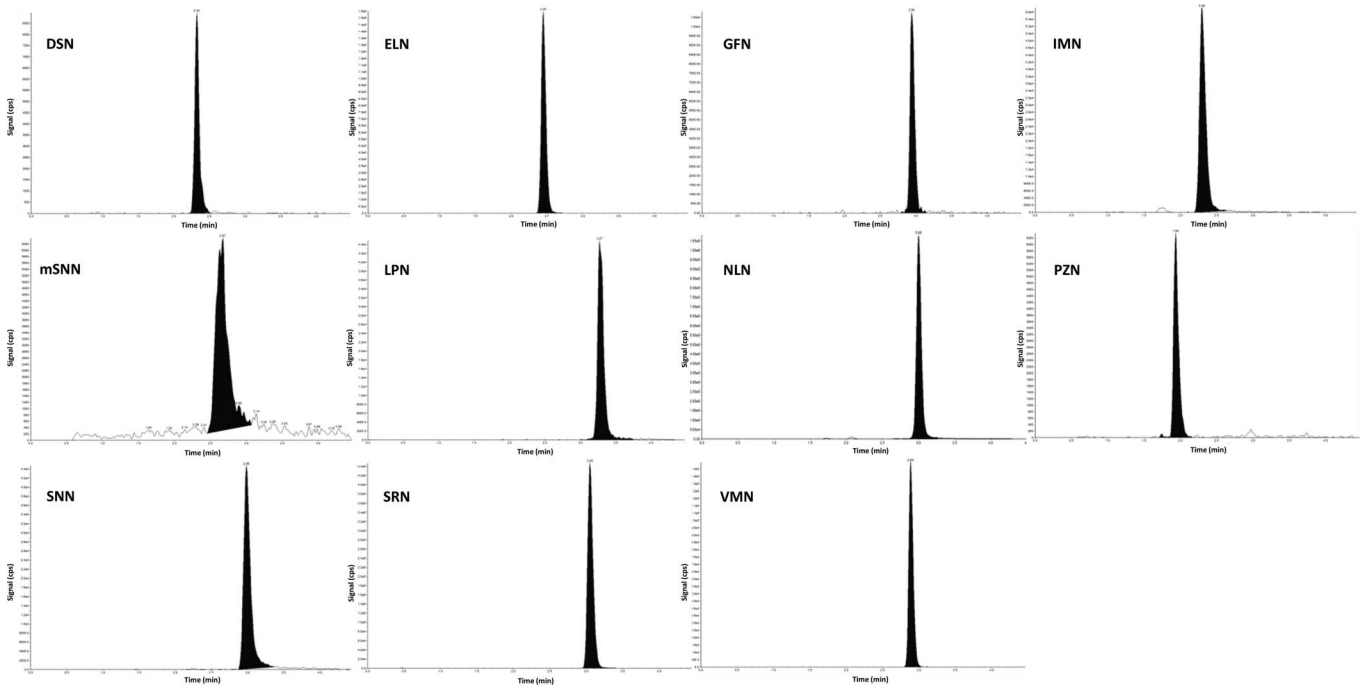

FIGURE 2. Chromatograms of analyzed patient samples [bracketed concentrations (ng/mL)] TKIs: DSN, dasatinib (57.2); ELN, erlotinib (1,790); GFN, gefitinib (498); IMN, imatinib (1,140); LPN, lapatinib (883); mSNN, N-desethyl-sunitinib (27); NLN, nilotinib (1,730); PZN, pazopanib (28,900); SNN, sunitinib (67.5); SRN, sorafenib (2,190); and VMN, vemurafenib (48,000).

3 different levels (LLOQ, MID, and ULOQ) instead of the 4 levels (LLOQ, LOW, MID, and HIGH) used previously. The developed assay was suited to the demands of clinical practice where accurate results are needed in a relatively short time.

All validated parameters complied with the FDA Guidelines that were adjusted to suit TDM use, except for the carryover of lapatinib. Therefore, lapatinib samples should be evenly distributed across the entire batch of samples. The stability results demonstrated that the developed assay had a workable set-up. The stock stability at 20–25°C was sufficient for the preparation of the working solutions and subsequent spiking of the plasma. The patient samples were adequately stable but may require reanalysis, which would result in additional freeze–thaw cycles. The validated 3-cycle process ensures this possibility. When needed, a full analytical run consisting of calibrators, QC samples, and patient samples can be reinjected up to 24 hours after the first run. The measured patient plasma concentrations of all analytes were within the validated range of the developed assay. This demonstrates that the developed method is suitable for the use in TDM practice.

CONCLUSIONS

A liquid chromatography/tandem mass spectrometry method was developed and validated for the simultaneous analysis of the TKIs: dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, pazopanib, sunitinib, sorafenib, and vemurafenib, as well as the metabolite N-desethyl-sunitinib in human plasma for TDM. The TKI- and metabolite-spiked human plasma was pretreated by protein precipitation with acetonitrile and the addition of stable isotope labeled internal standards. The developed assay is appropriate for use in TDM services.

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