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Research Article

Development and clinical application of a liquid chromatography-tandem mass spectrometry-based assay to quantify eight tyrosine kinase inhibitors in human plasma

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ABSTRACT

Introduction: Tyrosine kinase inhibitors (TKIs) are widely used in tumor treatment. The detection of these medicines by liquid chromatography-tandem mass spectrometry (LC-MS/MS) can avoid the interference of structurally similar compounds.

Objectives: This study aimed to develop and validate a new LC-MS/MS assay for the quantification of eight tyrosine kinase inhibitors in human plasma and to preliminarily evaluate the clinical utility of the therapeutic drug monitoring method.

Methods: Plasma samples were prepared by simple protein precipitation and separated using an ultra-high-performance reversed phase column. Detection was achieved using a triple quadrupole mass spectrometer in the positive ionization mode. The assay was validated against standard guidelines. We reviewed and analyzed the results of 268 plasma samples obtained from patients administered imatinib and other TKIs collected from January 2020 to November 2021 at Zhongshan Hospital. The analytes were separated and quantified within 3.5 min.

Results: The newly developed method demonstrated linearity for the detected drug concentration in the range of 20 to 2000 ng/ml for gefitinib ($r^2 = 0.991$) and crizotinib ($r^2 = 0.992$), 50 to 5000 ng/ml for nilotinib ($r^2 = 0.991$) and imatinib ($r^2 = 0.995$), 1500–150,000 ng/ml for vemurafenib ($r^2 = 0.998$), 1000–100,000 ng/ml for pazopanib ($r^2 = 0.993$), 0.5–100 ng/ml for axitinib ($r^2 = 0.992$) and 5–500 ng/ml for sunitinib ($r^2 = 0.991$) and N-desethyl sunitinib ($r^2 = 0.998$). The lower limit of quantification (LLOQ) was 20 ng/ml for gefitinib and crizotinib, 50 ng/ml for nilotinib and imatinib, 1500 ng/ml for vemurafenib, 1000 ng/ml for pazopanib, 0.5, and 5 ng/ml for sunitinib and N-desethyl sunitinib, respectively. Specificity, precision, accuracy, and stability were tested, and met the requirements of the guidelines. At the same dose, there was no significant difference in plasma drug concentration between the original imatinib medicine and the generic medicine after patent expiration.

Conclusion: We developed a sensitive and reliable method for the quantification of eight TKIs.

Introduction

Tyrosine kinase inhibitors (TKIs) are a group of pharmacological agents that disrupt the signal transduction pathways of protein kinases through several modes of inhibition. The advent of imatinib (the most representative medicine for TKIs) has presented a new era of targeted therapy for tumors [1]. TKIs are generally used in the treatment of several malignant diseases. For example, imatinib and nilotinib have been used in the treatment of chronic myeloid leukemia (CML) [1,2], vemurafenib for melanoma [3], gefitinib and crizotinib for non-small cell lung cancer (NSCLC) [4–6], axitinib and pazopanib for renal cell carcinoma (RCC) [7,8], and sunitinib for gastrointestinal stromal tumors

Abbreviations: TKIs, Tyrosine kinase inhibitors; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; CML, Chronic myeloid leukemia; NSCLC, Non-small cell lung cancer; RCC, Renal cell carcinoma; GIST, Gastrointestinal stromal tumors; TDM, Therapeutic drug monitoring; CV, Coefficient of variation.

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(GIST) [9].

Therapeutic drug monitoring (TDM) for TKIs is extremely important, as inappropriate concentrations of TKIs can lead to ineffective treatment or unendurable side effects, including folliculitis, paronychia, facial hair growth, facial erythema, edema, nausea, hypothyroidism, vomiting, diarrhea, anemia, thrombocytopenia, and neutropenia [10,11]. In addition, the cost of excessive medication can be a serious burden on patients and medical insurance. To our knowledge, several methods for TKI detection have been published. Parise et al. developed an HPLC-MS method for imatinib detection [12], and Alvarez et al. developed an LC-MS/MS method for the detection of vemurafenib [13]. However, many of the published methods can only detect one or two TKIs. In clinic, the combination of TKI drugs is more common and it's also more important to detect TKI drugs and their active forms in vivo, such as sunitinib and its metabolite. [14,15]To meet the clinical requirements for the detection of different types of TKIs and their metabolites, a more efficient method is urgently needed.

Here, we developed and validated a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for the measurement of gefitinib, nilotinib, pazopanib, vemurafenib, imatinib, axitinib, crizotinib, sunitinib and its metabolite N-desethyl sunitinib in human plasma. To take the isomer of sunitinib into consideration E- and Z-type sunitinib and N-desethyl sunitinib were all detected.

Materials and methods

Reagents and instruments

Gefitinib, nilotinib, pazopanib, vemurafenib, imatinib, axitinib, crizotinib, Z-sunitinib, and E-sunitinib were isolated by Waters® ACQUITY UPLC® I CLASS (Milford Waters, MA, USA) and quantified using SCIEX® TRIPLE QUAD^{TM} 5500(AB Sciex, CA, USA). Gefitinib, nilotinib, pazopanib, vemurafenib, imatinib, axitinib, crizotinib, Z-sunitinib, and e-sunitinib were purchased from Aladdin (Shanghai, China). N-desethyl sunitinib hydrochloride, gefitinib-D6, nilotinib-D3, pazopanib-[C13, D3] hydrochloride, vemurafenib-^{13}C6, imatinib-D8, axitinib-[C13, D3], crizotinib-[^{13}C2, D5], sunitinib-D4 and N-desethyl sunitinib-D5 hydrochloride were obtained from Shimatsu AlsaChim (Illkirch-Graffenstaden, France). High-performance liquid chromatography grade methanol and acetonitrile were purchased from Merck Millipore (Billerica, MA USA), formic acid from Roe Scientific (Newark, DE, USA) and DMSO from Aladdin (Shanghai China). Mass spectrometry-grade ammonium formate was purchased from Sigma-Aldrich (Darmstadt, Germany). Human blank plasma was collected from an abandoned blood sample of apparently healthy individualswhich were provided by the health examination center of Zhongshan Hospital Fudan University (Shanghai, China) (IRB_B2020-310).

Sample preparation

Preparation of stock and working solutions

The TKIs mentioned above are divided into two groups: Group A (imatinib, nilotinib, gefitinib, pazopanib, and vemurafenib) and Group B (sunitinib, N-desethyl sunitinib, axitinib, and crizotinib). All of the TKIs were prepared in DMSO to a stock concentration of 1 mg/ml. Then, the stock solutions of the two groups were mixed and diluted with acetonitrile/ddH2O (4:1, v/v) to obtain the solutions as calibrators. Another technician prepared quality controls (QCs) with acetonitrile/ddH2O (4:1, v/v) according to the concentration requirements. The isotope-labeled internal standard (IS) stock solutions were diluted with methanol as the IS working solution at different working concentrations.

Preparation of sample

The TKIs mentioned above were divided into two groups: Group A (imatinib, nilotinib, gefitinib, pazopanib, and vemurafenib) and Group B (sunitinib, N-desethyl sunitinib, axitinib, and crizotinib). The main difference between the pre-treatment of Groups A and B was the dilution ratio; all other conditions were consistent.

Group A: 5 μL of QC and calibrator were diluted with 95 μL of human blank plasma. The mixture was then vortexed for 2 min. Then, 20 μL of patient sample (or diluted QC and calibrator) was mixed with 180 μL of IS working solution and vortexed for 2 min. After centrifugation at 12,000×*g* for 5 min at room temperature, 20 μL of the supernatant was mixed with 380 μL of 0.1% FA in methanol: water (1:4, v/v), and 10 μL of the mixture was injected into the LC-MS system.

Group B: 5 μL of QC and calibrator was diluted with 95 μL of human blank plasma. The mixture was then vortexed for 2 min. Then, 100 μL of patient sample (or diluted QC and calibrator) was mixed with 400 μL of IS working solution and vortexed for 2 min. After centrifugation at 12,000×*g* for 5 min at room temperature, 100 μL of the supernatant was mixed with 100 μL of 0.1% FA in water, and 10 μL of the mixture was injected into the LC-MS system.

LC-MS/MS procedure

Separation was performed using Waters® ACQUITY UPLC® I CLASS, C18 50 mm × 2.1 mm, 1.7 μm (Milford Waters, MA, USA) at a temperature of 50 °C. The mobile phase consisted of 0.1% formic acid and 5 mM ammonium formate in ddH_2O (A) and acetonitrile (B). The flow rate was 0.4 ml/min and the elution gradient is shown in Supplemental Table 1.

The SCIEX® TRIPLE QUAD^{TM} 5500 was operated in the positive ion multiple reaction monitoring (MRM) mode using a turbo spray. The dwell time for each transition was 50 ms. The ion spray voltage, source temperature, collision gas (CAD), curtain gas (CUR), GS1, and GS2 were optimized at 5500 V, 500 ℃, 8 psi, 30 psi, 50 psi, and 50 psi, respectively. The other parameter settings are summarized in Supplemental Table 2.

Method validation

Validation of the method was performed according to the C62-A document generated from the Clinical and Laboratory Standards Institute (CLSI) [12], as well as the Chinese Guidance for Liquid Chromatography and Mass Spectrometry Clinical Application [16]. Calibration linearity, lower limit of quantification (LLOQ), specificity, accuracy, matrix effect, precision, and stability were all evaluated according to the above-mentioned guidelines.

Lower limit of quantification

The LLOQ is the lowest concentration that can be accurately detected while meeting the requirements for accuracy and precision. Serial dilutions with the lowest point of the calibrator standard sample gave the LLOQ for the nine analytes when the deviation of the detection value from the theoretical value was <15% and the coefficient of variation (CV) was within 15%. It should also be noted that the signal-to-noise ratio was >20:1 for the LLOQ. The limit of detection (LOD) was defined as the lowest concentration that can be detected with a signal-to-noise ratio >3:1, according to the guidelines. All samples were analyzed in duplicates during the same analysis.

Calibration linearity

Repeated testing of the calibrators assessed the linearity of the assay. Seven points were tested in total, with three replicates, and linearity was assessed using multiple regression analysis and linearity equations. The assay was considered linear when the Pearson correlation coefficient (r^2) was >0.99.

Specificity and matrix effect

To assess specificity, five samples at the LLOQ and five samples at the IS were tested. The background peak area to LLOQ or IS peak area ratios were calculated, and the specificity was validated when the background

peak area/LLOQ peak area was <5% and the background peak area/IS peak area was <1%. Human blank plasma and methanol were used to assess matrix effects. We examined the ratio of analyte response in methanol and blank human plasma to determine whether there was ion enhancement or inhibition. We evaluated the relative matrix effect of the target analytes at both high and low concentrations. We did not use an internal standard because the drugs we tested are exogenous substances that do not exist in human blank plasma.

Intra- and inter-assay precision

Intra-assay precision was evaluated by testing QC samples, which were diluted from standard solutions at three different concentrations, and each concentration was measured five times in one day. The QC levels for each compound are listed in Table 1. To evaluate the inter-assay precision, each concentration of the QC sample was tested three times on five different days. Both intra- and inter-assay precisions should meet the requirement that the CVs be within ±15%.

Recovery

An experiment to assess the accuracy of the assay was conducted. Three levels of quality control were added to human blank plasma, and these samples were tested five times; the measured values were compared to the expected value. This was considered acceptable when the measured value was within ±15% of the target value.

Stability

Stability was assessed by measuring the calibration solution, QC, and IS solutions under different conditions. Samples were stored at room temperature for 24 h, thawed-frozen three times at −80 °C, and the calibration solution, QC, IS solutions and samples were detected three times. Assessment was carried out for both QCL and QCH. The mean concentration deviation at each level was <15%.

Statistical analysis and clinical application

Patient whole blood samples which were collected to develop the assay, and to analyze patient samples with the developed assay were provided by Zhongshan Hospital Fudan University (Shanghai, China) (IRB_B2020-310). The results of 268 cases of imatinib and other TKI tests were collected from January 2020 to November 2021 at Zhongshan Hospital, including 35 pairs of trough and peak concentrations and 198 trough concentrations of imatinib. Based on the medication name (Glivec vs. Xinwei) and dosage (300 mg qd vs. 400 mg qd), all the results were divided into four groups: Glivec 400 mg qd (1a), Glivec 300 mg qd (1b), Xinwei (imatinib mesylate tablet made by HANSOH PHARMA, Jiangsu, China) 400 mg qd (2a), and Xinwei 300 mg qd (2b). The values of median, mean (2.5th percentile and 97.5th percentile), coefficient of variation (CV), and other statistical parameters were defined using SPSS version 20 (IBM, Chicago, IL, USA). The Mann-Whitney *U* test was used to evaluate the difference in imatinib concentrations between different doses and different kinds of medicines.

Results

Method development

Individual samples had a total run time of 3.5 min. All eight TKIs could be completely separated under the chromatographic conditions, particularly the two isomers of sunitinib. All the analytes of interest and the isotopic internal standard were in different MRM channels, and there was no crosstalk. The chromatogram and retention times for each detection are shown in Fig. 1.

Method validation

The calibration samples were calibrated at different concentrations

Table 1
Precision and accuracy of the LC-MS/MS assay.

Analyte	QC levels	Intra-day (n=5)		Inter-day (n=15)		Recovery (n=5)
		Mean	CV (%)	Mean	CV (%)	Mean (range, %)
Gefitinib(ng/ml)						
Low	64	54.6	6.58	58.08	7.24	91.00 (85–100)
Medium	320	311.58	6.57	321.45	6.59	97.37 (88–105)
High	1600	1572.13	5.82	1576.34	6.42	98.26 (90–107)
Nilotinib(ng/ml)						
Low	160	149.75	8.09	152.43	8.78	99.83 (92–113)
Medium	800	825.94	4.64	812.79	3.41	94.94 (87–100)
High	4000	3623.48	5.40	3730.86	4.25	90.59 (85–99)
Pazopanib (ng/ml)						
Low	3200	2970	6.13	3100	6.32	98.97 (91–103)
Medium	16,000	16,420	5.13	16,840	6.06	102.60 (97–110)
High	80,000	75,840	4.98	76,870	4.27	94.80 (90–103)
Vemurafinib (ng/ml)						
Low	4800	4610	7.70	4500	5.68	102.41 (90–112)
Medium	24,000	24,840	7.39	24,170	6.51	103.52 (88–109)
High	120,000	124,430	5.01	122,120	4.34	103.69 (98–112)
Imatinib(ng/ml)						
Low	160	145.54	4.71	144.03	5.2	97.03 (91–104)
Medium	800	812.14	7.46	796.09	5.18	101.52 (94–107)
High	4000	3870.13	3.57	3879.21	4.38	96.75 (92–101)
Axitinib(ng/ml)						
Low	1.6	1.38	5.90	1.40	6.72	91.77 (83–98)
Medium	8	8.68	4.77	9.06	6.72	86.80 (82–95)
High	80	77.02	5.47	75.49	6.73	96.27 (90–104)
Crizotinib (ng/ml)						
Low	64	61.37	7.82	59.97	8.52	102.28 (89–110)
Medium	320	313.88	3.73	317.95	4.79	98.09 (94–103)
High	1600	1554.89	3.40	1508.90	3.72	97.18 (92–100)
Sunitinib (ng/ml)						
Low	16	15.86	4.04	14.64	8.61	105.73 (99–111)
Medium	80	77.49	4.82	76.64	4.08	96.86 (92–105)
High	400	383.12	2.83	367.07	5.06	95.78 (93–101)
N-desethyl Sunitinib (ng/ml)						
Low	16	15.48	2.99	14.91	7.33	103.18 (99–107)
Medium	80	77.34	5.14	78.04	4.69	96.67 (90–103)

(*continued on next page*)

Table 1 (*continued*)

Analyte	QC levels	Intra-day (n=5)		Inter-day (n=15)		Recovery (n=5)
		Mean	CV (%)	Mean	CV (%)	Mean (range, %)
High	400	390.29	3.74	384.01	4.17	97.57 (94–102)

The mean and CV of intra-day (n = 5) and inter-day (n = 15) analyses are shown to evaluate the precision. The mean and range of recovery (n = 5) at three levels of analyte concentrations are shown to evaluate the accuracy.

of the seven levels for each analyte, and the calibration curves were linear with r^2 values for Gefitinib, nilotinib, pazopanib, vemurafenib, imatinib, axitinib, crizotinib, Z-sunitinib, and E-sunitinib of 0.991, 0.991, 0.993, 0.998, 0.995, 0.992, 0.992, 0.991 and 0.997 respectively (all $r^2 > 0.99$; Supplementary Fig. 1). The LLOQs and CVs of each analyte are listed in Table 2 and the LODs are also listed in Table 2 with 100% LOD detection for all nine analytes. All QC precision was within 15%. The ratios of background peak area/low limit peak area and background peak area/IS peak area were within 2.7% and 0.03%, respectively (Supplementary Table 3), indicating good specificity of the method. Matrix effects were assessed by adding low and high concentrations of pure standard to human blank plasma and methanol (Supplementary Table 3), with the ratio of analyte response of the TKIs in methanol and blank human plasma meeting the requirements and relative matrix effects being negligible. Both intra- and inter-assay precision met the requirement that the CVs should be within ±15%, with the highest CV values of intra-assay and inter-assay precision being 8.09% and 8.78%, respectively (Table 1), indicating that our method had good repeatability. Accuracy was evaluated using extraction and recovery experiments; sample average extraction recoveries for low-, medium-, and high-concentration samples ranged from 82% to 113% for all analytes (Table 1), in line with the requirement that recoveries should be between 85% and 115%. The stability of the working calibrator, QC, and IS solutions were validated with all biases <15% under different conditions (data not shown).

Clinical application

From January 2020 to November 2021, there were six tests of axitinib, four tests of pazopanib, and thirteen tests of sunitinib. Tests of vemurafenib, crizotinib, gefitinib, and nilotinib were not ordered during the study period. For TDM of these drugs, our study has yet to accumulate further data. Our main clinical test is imatinib, and we have conducted drug equivalence studies.

Four groups of cases were all tested using the Shapiro-Wilk test to assess the normality of the data, and only Group 2b showed normality. The Mann-Whitney test was used to test the difference between groups and a significant difference was only observed between Groups 2a and 2b. All data are shown in Table 3.

Discussion

Several LC-MS/MS assays have been described for the quantitation of TKIs [17–19]. However, to our knowledge, this is the first reported assay for quantification of eight TKIs using only 100 μL of plasma (20 μL of plasma for imatinib, nilotinib, gefitinib, pazopanib, and vemurafenib). The newly developed LC-MS/MS assay significantly improved the accuracy of the detection of these eight TKIs and our future goal is to study the relationship between plasma drug concentration and cancer prognosis. Our method was validated according to C62-A [20] and the Chinese Guidance for Liquid Chromatography and Mass Spectrometry Clinical Application [16], exhibiting good linearity, specificity, precision, accuracy, and stability. Furthermore, over the course of two years of operation of this method, approximately 300 patients have used it to monitor their TKI blood drug concentration and make medicine dosage adjustments.

There are several limitations to be noted in the development of the proposed method. Firstly, it must be taken into account that the drug concentration of these eight medicines in the body spans a wide range [3,21–23], and the drug concentration in the body is relatively high. At the same time, the mass spectrometer also has the problem of signal saturation; when the concentration was too high, the ionization efficiency decreased significantly. Therefore, we divided the eight medicines into two groups, using two different dilution methods, so that the linearity of the analytes could meet the requirements of TDM in patients' plasma. The E- and Z-types of sunitinib have the same ion pair, so the triple quadrupole mass spectrometer cannot be separated by different ion channels. At this time, chromatographic separation is required. The gradient elution of our chromatographic method successfully separated the cis and trans isomers of sunitinib and N-desethyl sunitinib within only 3.5 min.

The clinical application of TKIs also faces huge challenges owing to individual differences. For patients with the same disease and receiving the same dosage of medication, the individual difference reaches 10–20 times. Approximately 30% of patients are at a risk of insufficient medicine exposure and poor efficacy, while excessive exposure and risk of adverse reactions occurs in 15% of patients [24]. We collected TDM information from patients from laboratory information systems. All patients had blood taken before taking the drug for two weeks after the dose was stable to determine their stable trough concentration. Our data showed that there was no significant difference in plasma drug concentrations between the different dose groups (Glivec 400 vs. 300 mg qd), respectively. Glivec's patent protection expired in 2013, and the subsequent emergence of generic medicines in China reduced the heavy economic burden for CML and GIST patients. Our research shows that there is also no significant difference in plasma drug concentration between the reference listed medicine and the generic medicine at the same dose. Our results are consistent with the pharmacodynamic results of many generic drugs. [25,26] TDM of TKIs is a powerful tool for guiding individualized medication. Many studies have shown that adjusting the dosage according to the patient's plasma concentration can significantly improve a patient's prognosis [27,28]. Our method has been established for nearly two years and provides a powerful tool for patients who use TKIs to understand their personal situations after taking the medicine. Clinicians in our hospital adjust the dosage of patients through TDM results to avoid poor efficacy caused by insufficient medicine use and side effects caused by overdose.

Although we have established mass spectrometry methods for the eight TKIs and these have been in clinical application for nearly two years, several problems still need to be investigated and addressed. Some medicines whose main metabolites are not included in our method, such as the main metabolite of imatinib [29], may cause confusion in clinical evaluation. In our follow-up study, we will strengthen our cooperation with the Departments of Oncology, Surgery, and Hematology to study the relationship between plasma drug concentration and tumor prognosis, and provide strong evidence for the clinical use of TKIs.

Conclusions

We developed and validated a new LC-MS/MS assay for the quantification of eight TKIs. Our research found that there was no significant difference in plasma drug concentration between the reference listed medicine and the generic medicine at the same dose. The relationship between plasma drug concentration and tumor prognosis should be further explored in the future.

Author contributions

All authors have accepted responsibility for the entire content of this

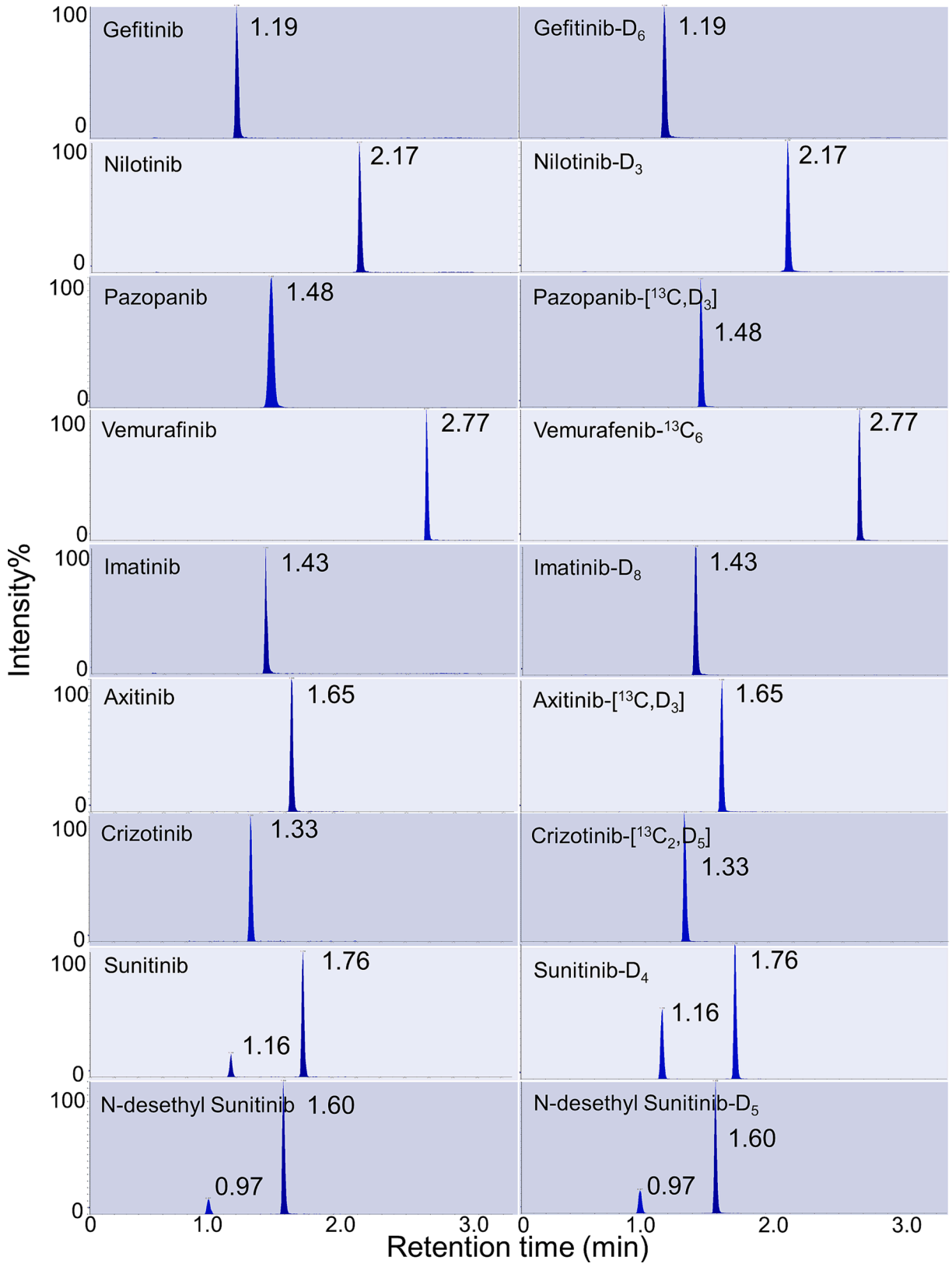

Fig. 1. A representative chromatogram showing signals of eight TKIs. The eight TKIs are gefitinib (A), nilotinib (B), pazopanib (C), vemurafenib (D), imatinib (E), axitinib (F), crizotinib (G), sunitinib (H), and N-desethyl sunitinib (I), with the retention times of 1.19 min, 2.17 min, 1.48 min, 2.17 min, 1.43 min, 1.65 min 1.33 min, 1.76 min and 1.60 min, respectively.

Table 2
Method validation results including calibration range, lower limit of quantification (LLOQ) and limit of detection (LOD) of each TKI.

Analyte	Calibration range (ng/ml)	LLOQ (n=10)	CV (%) (n=10)	Average deviation (%) (n=10)	Limit of detection (LOD) (n=10)	
					LOD	LOD detection rate (%)
Gefitinib	20–2000	20	14.61	−2.05	10	100
Nilotinib	50–5000	50	5.59	−0.55	25	100
Pazopanib	1000–100,000	1000	5.72	−1.65	500	100
Vemurafinib	1500–150,000	1500	9.60	4.84	750	100
Imatinib	50–5000	50	13.60	−9.65	25	100
Axitinib	0.5–100	0.5	8.71	−11.33	0.25	100
Crizotinib	20–2000	20	4.29	3.53	10	100
Sunitinib	5–500	5	6.93	−1.73	2.5	100
N-desethyl Sunitinib	5–500	5	11.87	10.46	1.25	100

Table 3
The plasma concentration of Imatinib in all patients was divided into four groups according to the type and dose of the drug. All patients had blood taken before taking the drug for two weeks after the dose was stable to determine their stable trough concentration. Group 1a: Glivec 400 mg qd, Group 1b: Glivec 300 mg qd, Group 2a: Xinwei 400 mg qd, Group 2b: Xinwei 300 mg qd. All values are shown as ng/ml. All differences according to the Mann-Whitney *U* test.* $P < 0.05$ different from specific group.

Group	n	mean(2.5th-97.5th)	SD	S-W test Sig.	M-W test Sig.
1a	59	1653.52 (556.22–5070.85)	901.09	0.000	——
1b	26	1383.75 (628.84–2589.60)	637.43	0.018	0.179(with 1a)
2a	91	1558.01 (425.4–4544.69)	883.02	0.000	0.276(with 1a)
2b	57	1206.99 (337.29–2281.72)	449.52	0.612*	0.426(with 1b) 0.037* (with 2a)

submitted manuscript and approved submission. W.G. created the study concept. W.G. and B.W. contributed to the study design F.C., W.C., and Z.W. contributed to the method development, method validation, and analysis of the data. F.C. and Y.P. contributed to data acquisition and collection of clinical samples. F.C. and W.C. contributed to the drafting of the manuscript. W.G. contributed to the critical revision of the manuscript.

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Employment or leadership

None declared.

Honorarium

None declared.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2023.05.001.

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