Drug Metabolism and Pharmacokinetics 31 (2016) 12–20

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Review

Routine therapeutic drug monitoring of tyrosine kinase inhibitors by HPLC–UV or LC–MS/MS methods

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ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form
14 September 2015
Accepted 20 September 2015
Available online 12 November 2015

Keywords:
Imatinib
Nilotinib
Dasatinib
HPLC
LC–MS/MS

ABSTRACT

Analytical methods using high performance liquid chromatography coupled to ultraviolet detection (HPLC–UV) or liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been reported for the quantification of oral tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib, and dasatinib in biological fluids. An LC–MS/MS method can simultaneously assay multiple TKIs and their metabolites with high sensitivity and selectivity for low plasma concentrations less than 1 ng/mL. For quantification of imatinib, nilotinib, and dasatinib, a limit of quantification (LOQ) of less than 10 ng/mL, 10 ng/mL, and 0.1 ng/mL, respectively, in the clinical setting is necessary. Because simpler and more cost-efficient methodology is desired for clinical analysis, plasma concentrations of imatinib and nilotinib (target trough concentrations of 1000 ng/mL and 800 ng/mL, respectively) could be assayed by an HPLC–UV method after comparison with results obtained from the standard LC–MS/MS method. However, in the quantification of dasatinib, the LC–MS/MS method that has high sensitivity and selectivity and is free from interference by endogenous impurities is superior to the HPLC–UV method. Highly precise analytical methods are needed for individualized treatment via dose adjustment of oral anticancer drugs, in particular those with low target plasma concentrations less than 10 ng/mL.

Contents

1. Introduction

Tyrosine kinase inhibitors (TKIs) such as imatinib, nilotinib, and dasatinib are oral anticancer drugs that inhibit the adenosine triphosphate binding site of tyrosine kinase receptors in malignant cells. Recently, the importance and necessity for therapeutic drug monitoring (TDM) of these TKIs has been demonstrated [1–7]. TDM is carried out by evaluating drug plasma concentrations to provide individual treatment through dose-adjustment to avoid adverse events and to transition from an insufficient response from underdosing to a clinical effect. For imatinib, the relationship between plasma concentration and clinical response has been

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http://dx.doi.org/10.1016/j.dmpk.2015.09.002

observed [1–3,5,8–13]. In Japan, a treatment fee (health care fee) for managing the TDM of imatinib for patients with Philadelphia chromosome-positive chronic myeloid leukemia (CML) or gastrointestinal stromal tumors (GIST) has been assessed since 2012 [3,5]. To carry out TDM, although therapeutic target ranges indicate relationships between exposure and response (the minimum effective concentration (MEC) is the concentration required to produce a desired pharmacological effect and the minimum toxic concentration (MTC) is the concentration that produces toxic effects in most patients) must be determined, a trough concentration of 1000 ng/mL has been targeted as the efficacious concentration for imatinib [1,4,10,14–17]. However, especially in younger patients, 2nd generation TKIs nilotinib or dasatinib rather than imatinib are preferred, because they achieve a more robust molecular response and eventually achieving treatment-free remission is strongly expected [18]. Similar to imatinib, TDM of nilotinib or dasatinib might also be useful for cancer therapy [5,17]. At least, striving to avoid therapeutic failure and unnecessary costs by long-term transition from lower TKI exposure should be clinical goals. Therefore, it is necessary to regularly obtain information about the plasma exposure of orally administered TKIs.

Currently, analytical methods using high performance liquid chromatography coupled to ultraviolet detection (HPLC–UV) or liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been reported for the quantification of these TKIs in biological fluids. In particular, the LC–MS/MS method can simultaneously assay multiple TKIs and their metabolites. However, because TDM is routinely carried out, the high purchase cost, high maintenance cost, and running costs of LC–MS/MS limit accessibility in research laboratories, and there are few standard hospital laboratories that use LC–MS/MS. On the other hand, clinicians cannot regularly monitor plasma concentrations of imatinib because of the costs of using outside research laboratories, and consequently TDM has not been adapted in the clinical setting. Since it is important to consider the costs and availability of analytical instruments, one major benefit of assaying by HPLC–UV is its availability in hospitals and small laboratories in comparison to the high cost of the LC–MS/MS apparatus. Therefore, if accuracy, precision, and sensitivity for the quantification of a TKI by an HPLC method are equivalent to the LC–MS/MS method, HPLC–UV would represent a superior methodology.

The aim of this paper is to review the current knowledge on analytical methods for TDM and clinical studies on exposure–response relationships of TKIs such as imatinib, nilotinib, and dasatinib.

2. Quantification of imatinib

HPLC–UV (Table 1) and LC–MS/MS (LC–MS) (Table 2) assays to quantify the total imatinib concentration in human plasma and serum have been developed. All HPLC–UV methods show intra-day and inter-day coefficients of variation (CV) less than 20% in the concentration range of the calibration curve (Table 1) [19–30]. Ultraviolet (UV) sample detection is carried out in the wavelength range of 260–270 nm [19–26,28–30]. The limit of quantification (LOQ) of imatinib for each method ranged from 2 ng/mL to 100 ng/mL. These analytical HPLC methods require relatively large sample volumes (300–750 μL) to achieve adequate sensitivity [19,21,25,28,29]. Clinically, it should be considered that the blood volume collected from children is limited, and each sample analysis needs to be performed in duplicate. Furthermore, in the quantification of the imatinib concentration, an internal standard, which is a compound chosen to not be used together in the clinic (for example, the same CML therapeutic agent, dasatinib and nilotinib), should be used in biological fluids. For drug quantification in human plasma samples, the addition of an internal standard is essential because errors at the lower end of the concentration range are minimized. If these problems could be overcome, then the HPLC method would be better from a cost perspective for assays than the LC–MS/MS method. The steady-state plasma trough concentrations (C_0) of imatinib after administration of a 400 mg standard daily dose and 300 mg in CML and GIST patients ranged from 109 ng/mL to 4980 ng/mL [1–3,9,10,14,15,31–39] and from 360 ng/mL to 2140 ng/mL, respectively [3,10,37–39]. Because there are CML and GIST patients that take a low daily dose of 100 mg or 200 mg imatinib or who have poor adherence to imatinib treatment, a LOQ less than 50 ng/mL of imatinib would appear to be desirable. An HPLC–UV assay developed by Oostendorp et al. and Miura et al. could be applied more widely for clinical analysis [22,26]. In particular, the HPLC–UV assay by Oostendorp et al. can simultaneously evaluate the concentrations of imatinib and its active metabolite (*N*-desmethyl imatinib) [22]. Presently, the

Table 1
HPLC–UV methods for the quantitation of imatinib in human plasma.

Reference	Year	Analyte(s)	IS	UV	Calibration range (ng/mL)	LOQ (ng/mL)	CV (%)	Extraction	Conc. rate	Sample volume (μL)
Schleyer E et al. [19]	2004	Imatinib, N-DI	–	260	10–20,000	No data 10 (LOD)	<8.6	LLE	1.3	270
Velpandian T et al. [20]	2004	Imatinib	–	265	25–25,000	30[a]	<4.9	LLE	0.5	100
Widmer N et al. [21]	2004	Imatinib	Clozapine	261	100–10,000	50[b]	<2.4	SPE	4.0	750
Oostendorp RL et al. [22]	2007	Imatinib, N-DI	4-Hydroxy-benzophenone	265	10–10,000	10[b]	<7.8	LLE	1.0	100
Davies A et al. [23]	2010	Imatinib, nilotinib, N-DI	Clozapine	260	100–12,000	50[b]	<4.53	SPE	1.0	200
Roth O et al. [24]	2010	Imatinib	–	265	80–4000	80[b]	<2.7	LLE	0.5	200
Awidi A et al. [25]	2010	Imatinib	Risperidone	265	100–4000	100[No data]	<4.22	LLE	2.5	500
Miura M et al. [26]	2011	Imatinib	Dasatinib	265	10–5000	10[b]	<11.9	SPE	0.75	100
Tan KL et al. [27]	2011	Imatinib, N-DI	Pyrilaminemaleate	235	50–1800	10[b]	<0.28	LLE	1.0	200
Pirro E et al. [28]	2011	Imatinib, dasatinib	Nilotinib	267	5–10,000	50[b]	<19.87	LLE–SPE	1.9	500
Golabchifar AA et al. [29]	2011	Imatinib, N-DI	Olanzapine	261	62.5–6000	62.5[b]	<12.4	LLE	1.9	300
Birch M et al. [30]	2013	Imatinib, N-DI	Norclomipramine	270	50–10,000	2[c]	<9	LLE	1.8	100

IS; internal standard, UV; ultra-violet, LOQ; limits of quantitation, LOD; limits of detection, CV; coefficient of variations (included intra-day and inter-day), N-DI; N-desmethyl imatinib, LLE; liquid–liquid extraction, SPE: solid-phase extraction, Conc. rate; concentration rate = plasma sample volume*recovery/pre-injecting sample volume.
[a] Standard deviation/slope of calibration curve.
[b] 20%CV value.
[c] 5 times the baseline noise.

Table 2
LC–MS/MS (LC–MS) methods for the quantitation of imatinib in human plasma/serum.

Reference		Year	Analyte(s)	IS	Calibration range (ng/mL)	LOQ (ng/mL)	CV (%)	Extraction	Conc. rate	Sample volume (μL)
Bakhtiar R et al. [40][a]	A	2002	Imatinib, N-DI	D8-imatinib	4.00–10,000	4.00[b]	<5.97	LLE	0.7	200
Parise RA et al. [41]	B	2003	Imatinib, N-DI	D8-imatinib	30–10,000	30[No data]	<7.2	LLE	2.0	200
Guetens G et al. [42]	A	2003	Imatinib	D8-imatinib	1–10,000	1.8[b]	<6.93	LLE	0.7	250
Titier K et al. [43]	A	2005	Imatinib	D8-imatinib	10–5000	10[b]	<7.46	LLE	1.8	200
Rochat B et al. [44]	A	2008	Imatinib	D8-imatinib	1.0–5000	1[b]	<13.6	LLE	1.8	100
De Francia S et al. [45]	B	2009	Imatinib, nilotinib, dasatinib	Quinoxaline	78.1–10,000	78.1[b]	<12.6	LLE	0.3	250
Chahbouni A et al. [46]	A	2009	Imatinib, erlotinib, gefitinib	D8-imatinib	5–5000	5[No data]	<4.6	LLE	1	100
Haouala A et al. [47]	A	2009	Imatinib, other 5 TKIs	D8-imatinib	1–10,000	1[b]	<6.8	LLE	0.2	100
Mičová K et al. [48]	A	2010	Imatinib, N-DI	D8-imatinib	100–5000	4[c]	<5.72	LLE	0.1	20
Awidi A et al. [25]	A	2010	Imatinib	D8-imatinib	10–4000	10[No data]	<12.01	LLE	0.4	200
Streit F et al. [49]	A	2011	Imatinib, N-DI	D8-imatinib	8.4–8370	8.4[No data]	<12.3	LLE	0.3	50
Bouchet S et al. [50]	A	2011	Imatinib, N-DI, Other 8 TKIs	D8-imatinib	10–5000	10[b]	<9.3	SPE	2.7	300
Kralj E et al. [51]	A	2012	Imatinib, nilotinib, dasatinib	D8-imatinib	50–5000	50[b]	<9.1	LLE	0.8	200
Zhang M et al. [52]	A	2012	Imatinib, N-DI	D8-imatinib	10–2000	10[b]	<7.4	LLE	0.2	50
Götze L et al. [53]	A	2012	Imatinib, other 5 TKIs	D8-imatinib	50–5000	? (3.8–12.6)[c]	<10.3	LLE	0.2	100
Couchman L et al. [54]	A	2012	Imatinib, N-DI, other 7 TKIs	D8-imatinib	50–5000	? < 10[d]	<8.22	LLE	0.3	50
Rezende VM et al. [55]	B	2013	Imatinib, N-DI	D8-imatinib	500–10,000	500[b]	<5.5	LLE	0.2	100
Lankheet NA et al. [56]	A	2013	Imatinib, other 7 TKIs	D3-imatinib	20–10,000	20[b]	<5.5	LLE	0.4	50
van Erp NP et al. [57]	A	2013	Imatinib, N-DI, other 5 TKIs	^{13}C3–^{15}N-nilotinib	100–4000	100[d]	<5.7	LLE	0.1	50
Andriamanana I et al. [58]	A	2013	Imatinib, other 8 TKIs	D8-imatinib	50–3500	50[b]	<7.3	LLE	0.2	50
Birch M et al. [30]	A	2013	Imatinib, N-DI, dasatinib	D8-imatinib	50–10,000	3[d]	<13	LLE	0.3	50
Zhang Y et al. [59]	A	2014	Imatinib, N-DI	Palonosetron	8–5000	8[b]	<2.15	LLE	0.3	400

A; LC–MS/MS, B; LC–MS, IS; internal standard, LOQ; limits of quantitation, CV; coefficient of variations (included intra-day and inter-day), N-DI; N-desmethyl imatinib, LLE; liquid–liquid extraction, SPE: solid-phase extraction, Conc. rate; concentration rate = plasma sample volume*recovery/pre-injecting sample volume.
[a] Global standard.
[b] 20%CV value.
[c] Signal-to-noise ratio of 10.
[d] Signal-to-noise ratio of 5.

plasma concentration of *N*-desmethyl imatinib is not included in the target imatinib C_0 of 1000 ng/mL. However, exposure to both imatinib and *N*-desmethyl imatinib could be considered.

The LC–MS/MS method of Bakhtiar et al. is recommended as the global standard for quantification of imatinib concentration (Table 2) [40], as any newly developed LC–MS/MS or HPLC–UV analytical method for the quantification of imatinib concentration is compared with the results obtained from the LC–MS/MS method of Bakhtiar et al. [40]. Overestimations or underestimations of the concentrations measured with other analytical methods compared to the standard LC–MS/MS method should be carefully scrutinized.

LC–MS/MS assays reported by 2014 have shown intra-day and inter-day CV of less than 20% for each calibration range (Table 2) [25,40–59]. The LOQ values for imatinib in most LC–MS/MS methods range from 1 ng/mL to 10 ng/mL using sample volumes of 50–200 μL [25,30,40,43,44,46–49,52–54], and D8-imatinib is used as an internal standard. Thus, the reported LC–MS/MS methods have a higher sensitivity (LOQ: approximately 1–10 ng/mL) than HPLC–UV methods (LOQ: approximately 10 ng/mL). In addition, LC–MS/MS methods have recently been reported to be able to simultaneously assay the concentrations of imatinib and other 5–8 oral TKIs such as nilotinib, dasatinib, and sunitinib [45,50,51,53,54,56–58]. When the availability of LC–MS/MS apparatus in laboratories is considered, it might be of interest to analyze multiple TKIs using a single assay system.

Relationships between imatinib C_0 and clinical response have been evaluated in several clinical trials of patients with CML and GIST using LC–MS/MS or HPLC–UV methods (Table 3) [1–3,8–13,36,39,60–63]. In many of these clinical trials, the LC–MS/MS method of Bakhtiar et al. was generally used for quantification of imatinib concentrations [2,3,9,10,39,62,63]. On the other hand, Gotta et al. monitored the imatinib C_0 using 3 different LC–MS/MS methods [24,47,50] for routine care in 2 clinical reports [64,65]. We routinely monitor imatinib plasma concentrations using the HPLC–UV method of Miura et al. [26,66]. The plasma concentrations of imatinib obtained by the HPLC–UV method of Miura et al. were almost the same as those assayed by the LC–MS/MS method of Bakhtiar et al. ($y = 1.0495x - 34.307$, $r^2 = 0.9842$, y: HPLC–UV, x: LC–MS/MS) [26]. This HPLC–UV method for assaying imatinib costs about $4 per sample including running cost.

A prospective trial (I-COME trial) reported by Gotta et al. evaluated whether TDM can improve the long-term response in CML patients [64]. In the I-COME trial, dose adjustment to accomplish a target imatinib C_0 of 1000 ng/mL (target range 750–1500 ng/mL) was performed in the routine TDM group; however, unfortunately, this trial did not show a benefit from routine TDM [64]. Clinical studies in CML patients by Forrest et al., Faber et al., and Racil et al. have also reported no significant difference in imatinib C_0 between responders and nonresponders (Table 3) [36,60,61]. However, several studies have reported that the imatinib C_0 for responders in imatinib therapy was significantly higher for non-responders

Table 3
Correlation of imatinib trough plasma concentration with clinical response.

Reference (method ref.)	Year	No	Response	Responders		Non-responders		*P*-value
				N	Mean C_0 (SD) (ng/mL)	N	Mean C_0 (SD) (ng/mL)	
CML								
Picard S et al. [1] (Titier, LC–MS/MS) [43]	2007	68	CCyR	56	1123 (±617)	12	694 (±556)	0.03
			MMR	34	1452 (±649)	34	869 (±427)	0.001
Larson RA et al. [2] (Bakhtiar, LC–MS/MS) [40]	2008	351	CCyR	297	1009 (±544)	54	812 (±409)	0.01
Singh N et al. [8] (Velpandian, HPLC) [20]	2009	40	Clinical response	20	2340 (±520)	20	690 (±150)	0.002
Sakai M et al. [9] (Bakhtiar, LC–MS/MS) [40]	2009	33	Optimal	25	1242	8	736	0.0087
Takahashi N et al. [3] (Bakhtiar, LC–MS/MS) [40]	2010	254	CCyR	218	1057 (±585)	36	835 (±524)	0.033
			MMR	166	1107 (±594)	88	873 (±528)	0.002
Ishikawa Y et al. [10] (Bakhtiar, LC–MS/MS) [40]	2010	60	MMR	38	1093 (median)	22	853 (median)	0.002
Awidi A et al. [11] (?, LC–MS/MS)	2010	103	MMR	49	2766 (±811)	54	1777 (±624)	0.001
Yoshida C et al. [39] (Bakhtiar, LC–MS/MS) [40]	2011	38	CCyR	33	977 (±605)	5	993 (±631)	0.48
			MMR	27	1044 (±636)	11	818 (±488)	0.17
			CMR	8	1430 (±988)	30	859 (±389)	0.04
Koren-Michowitz M et al. [12] (Parise, LC–MS) [41]	2012	147	CCyR	131	1078 (±545)	16	827 (±323)	0.045
Golabchifar AA et al. [13] (Golabchifar, HPLC) [29]	2014	60	MMR	31	1505	29	1117	0.004
Forrest DL et al. [60] (?, LC–MS/MS)	2009	78	CCyR	53	1010 (±469)	24	1175 (±656)	0.29
			MMR	51	1067 (±473)	27	1063 (±643)	0.74
Faber E et al. [36] (*Mičová*, LC–MS/MS) [48]	2012	84	CCyR	77	924 (median)	6	1481 (median)	ns
			MMR	60	998 (median)	24	900 (median)	ns
Racil Z et al. [61] (included, LC–MS/MS) [61]	2014	35	Optimal	27	829 (median)	8	1004 (median)	0.724
GIST								
Demetri GD et al. [62] (Bakhtiar, LC–MS/MS) [40]	2009	73	CR, PR, SD	57	1446 (median)	16	1155 (median)	0.25
Yoo C et al. [63] (Bakhtiar, LC–MS/MS) [40]	2013	52	PR, SD	31	3444 (±1724)	21	3713 (±1433)	0.56

N; number, C_0; trough concentration, ns; no significant difference between 2 groups.
CCyR; complete cytogenetic response, MMR; major molecular response, CMR; complete molecular response.
CR; complete response, PR; partial response, SD; stable disease.

[1–3,8–13,39]. In particular, five of these clinical studies utilizing the LC–MS/MS assay of Bakhtiar et al., showed the mean imatinib C_0 in responders and nonresponders ranged from 977 ng/mL to 1430 ng/mL and from 736 ng/mL to 993 ng/mL, respectively, which were common and reproducible results [2,3,9,10,39]. However, even if the LC–MS/MS method of Bakhtiar et al. was used for the imatinib assay, dose adjustment according to the target concentration should not be evaluated with a single imatinib C_0 data point, for example day 22, 28, or 29 after beginning imatinib treatment, because variability of the intra-patient CV for imatinib C_0 is very large ranging from 8.4% to 49.3% [17]. Consequently, the imatinib C_0 might be measured each week for the first month after beginning treatment, and then once a month to month three [5]. Three months after beginning imatinib therapy, non-responders with a mean imatinib C_0 less than 1000 ng/mL should have their dosage increased from 400 mg to 600 mg (or 500 mg) once daily after checking for compliance of imatinib usage or they should be switched to a 2nd generation TKI such as nilotinib and dasatinib. The mean imatinib C_0 for three months or using multiple points should be used to plan a treatment strategy for the subsequent three months [5]. Therefore, a highly precise methodology is required to provide accurate data for patient treatment, and a simpler and more cost-efficient methodology could be beneficial.

For CML patients, the target imatinib C_0 is typically set above 1000 ng/mL. Namely, analytical methods with a LOQ of 10 ng/mL for imatinib could be carried out routinely for TDM of CML and GIST patients. As hospital managers as well as analysts, consideration of cost performance is also important for analyses.

3. Quantification of nilotinib

Assays for nilotinib in human plasma or serum by HPLC–UV are shown in Table 4. These HPLC–UV methods have intra-day and inter-day CV of less than 20% in the calibration range, and UV detection is set in the wavelength range of 250–267 nm [23,28,67–69]. The LOQ values of these reported methods for nilotinib range from 5 ng/mL to 250 ng/mL. Clinically, an internal standard should be added, and large sample volumes (500 μL) should be avoided. In the East Japan CML (EJCML) study of 30 Japanese patients with imatinib-resistant or -intolerant CML, the

Table 4
HPLC–UV methods for the quantitation of nilotinib in human plasma.

Reference	Year	Analyte(s)	IS	UV	Calibration range (ng/mL)	LOQ (ng/mL)	CV (%)	Extraction	Conc. rate	Sample volume (μL)
Pursche S et al. [67]	2007	Nilotinib	–	258	5–5000	5[a]	<9.23	LLE	1.2	300
Davies A et al. [23]	2010	Nilotinib, imatinib, N-DI	Clozapine	260	100–12,000	50[a]	<4.73	SPE	1.0	200
Miura M et al. [68]	2010	Nilotinib	Dasatinib	250	10–5000	10[a]	<10.0	SPE	0.75	100
Yuki M et al. [69]	2011	Nilotinib	–	266	250–5000	250[No data]	<9.1	LLE	0.75	150
Pirro E et al. [28]	2011	Nilotinib, dasatinib	Imatinib	267	5–10,000	50[a]	<16.12	SPE	2.0	500

IS; internal standard, UV; ultra-violet, LOQ; limits of quantitation, CV; coefficient of variations (included intra-day and inter-day), N-DI; N-desmethyl imatinib, LLE; liquid–liquid extraction, SPE: solid-phase extraction, Conc. rate; concentration rate = plasma sample volume*recovery/pre-injecting sample volume.
[a] 20%CV value.

nilotinib C_0 ranged from 128 ng/mL to 2982 ng/mL [70,71]. In addition, in routine quantification of nilotinib in patient plasma, the nilotinib C_0 ranged from 16.8 ng/mL to 3525 ng/mL [72]. Patients with low plasma concentrations suggest the possibility of poor adherence to treatment [5]. The evaluation of nilotinib C_0 for adherence to treatment requires multiple C_0 values before the evaluation time point [5]. For evaluation of patients with poor compliance, analytical methods with a nilotinib LOQ of 10 ng/mL might be optimal. Applicable analytical methods for routine clinical practice should be developed. Consequently, the HPLC–UV assay developed by Miura et al. could be more widely applied clinically [68]. The LC–MS/MS method of Yin et al. is recommended as the global standard for quantification of nilotinib concentration (Table 5) [73]. The plasma concentrations of nilotinib obtained by the HPLC–UV method of Miura et al. [68] are almost the same as those assayed by the LC–MS/MS method of Yin et al. ($y = 1.0034x + 44.972$, $r^2 = 0.7971$, y: HPLC–UV, x: LC–MS/MS) [73]. Similar to imatinib, this HPLC–UV method could also be used to assay nilotinib with a low cost of approximately $4 per sample including running cost by changing the mobile phase of 0.5% KH_2PO_4 (pH 3.5) – acetonitrile–methanol (55:25:20,v/v/v) in the imatinib assay [26] to 0.5% KH_2PO_4 (pH 2.5) – acetonitrile–methanol (55:25:20,v/v/v) [68].

LC–MS/MS (LC–MS) methods for nilotinib assays in human plasma or serum are shown in Table 5 [45,47,50,51,53,54,56–58,73,74]. Two LC–MS/MS methods and one LC–MS method [47,73,74] have a higher sensitivity (LOQ: 1–5 ng/mL) than the HPLC–UV method [68] (LOQ: 10 ng/mL) and generally use sample volumes of 100–200 μL. In particular, the LC–MS/MS method developed by Haouala A et al. could be used to quantify both nilotinib and imatinib with a LOQ of 1 ng/mL using sample volumes of 100 μL and can simultaneously determine the concentrations of four other TKIs [47].

In the EJCML study, the nilotinib C_0 tended to be high in patients who achieved a major molecular response (MMR) after 12 months, but this result was not statistically significant. The median C_0 for patients who achieved MMR and those who did not achieve MMR were 774 ng/mL and 490 ng/mL (less than 500 ng/mL), respectively [71]. In the study of patients with imatinib-resistant or -intolerant CML, patients with a nilotinib C_0 less than 500 ng/mL had a significantly longer time to a complete cytogenetic response (CCyR, $P = 0.010$) and MMR ($P = 0.012$) [75]. In addition, in common practice, the steady-state mean nilotinib C_0 and the mean daily dose during the maintenance phase after 3 months of nilotinib therapy were approximately 800 ng/mL and 600 mg/day, respectively [72]. Based on the EJCML study [71] and another clinical study [72], a target nilotinib C_0 of 800 ng/mL is recommended for TDM [5]. However, intra-individual variation of nilotinib is greater than that of imatinib (mean CV value: 36.4% vs. 24.1%) [5,17], because the pharmacokinetics of nilotinib are affected by the ingestion of food. Therefore, similar to imatinib, the nilotinib C_0 after beginning 300 mg twice daily is recommended to be measured each week for the first month after beginning treatment and then monthly until month three, and the mean nilotinib C_0 using multiple points should be used to plan the subsequent treatment strategy [5]. In particular, therapeutic failure from long-term transition to lower nilotinib exposure should be avoided.

The nilotinib plasma concentration could also be assayed by the HPLC–UV method after comparison with the results obtained from the LC–MS/MS methods of Yin et al., since the nilotinib plasma concentration ranges between 10 and 4000 ng/mL.

4. Quantification of dasatinib

Two HPLC–UV methods for the quantitation of dasatinib in human plasma have been reported (Table 6) [26,28]. The HPLC–UV method of Pirro et al. had a LOQ of 100 ng/mL, although it required large sample volumes (500 μL) [28]. In a Phase III study, the mean steady-state dasatinib C_0 after taking 100 mg once daily was 2.61 ng/mL [76]. The steady-state geometric mean dasatinib C_0 in East Asian and non-East Asian patients taking 100 mg once daily were 2.11 ng/mL (n = 59, CV: 78%) and 1.54 ng/mL (n = 176, CV: 60%) [77]. In this report, the rate of pleural effusion was higher in East Asian patients than in non-East Asian patients (24% vs. 10%, respectively) [77]. Pleural effusion was also reported to be significantly associated with the dasatinib C_0, with the hazard ratio increasing 1.22-fold for every 1.0 ng/mL of C_0 increase, whereas the major cytogenetic response was significantly associated with dasatinib exposure [76]. In addition, in the prospective optimized tyrosine kinase inhibitors monotherapy (OPTIM) dasatinib trial, Rousselot et al. carried out TDM with a target dasatinib C_0 of 3 nM (1.5 ng/mL), and consequently, the overall rates of dasatinib-induced pleural effusion utilizing TDM during 36 months was reported to decrease from 48.9% to 11.3% [78]. Thus, a higher dasatinib

Table 5
LC–MS/MS (LC–MS) methods for the quantitation of nilotinib in human plasma/serum.

Reference		Year	Analyte(s)	IS	Calibration range (ng/mL)	LOQ (ng/mL)	CV (%)	Extraction	Conc. rate	Sample volume (μL)
De Francia S et al. [45]	B	2009	Nilotinib, imatinib, dasatinib	Quinoxaline	62.5–8000	62.5[b]	<19.2	LLE	0.3	250
Parise RA et al. [74]	B	2009	Nilotinib	$^{13}C_2$–$^{15}N_2$-nilotinib	5–5000	5[c]	<6.1	LLE	1.7	200
Haouala A et al. [47]	A	2009	Nilotinib, other 5 TKIs	D8-imatinib	1–5000	1[b]	<8.7	LLE	0.2	100
Yin OQ et al. [73][a]	A	2010	Nilotinib	$^{13}C_2$–$^{15}N_2$-nilotinib	7.5–4000	2.5[No data]	<7.2	LLE	No data	100
Bouchet S et al. [50]	A	2011	Nilotinib, other 8 TKIs	$^{13}C_2$–$^{15}N_2$-nilotinib	10–5000	10[b]	<10.0	SPE	2.7	300
Götze L et al. [53]	A	2012	Nilotinib, other 5 TKIs	D3-sorafenib	50–5000	? (3.8–12.6)[c]	<8.6	LLE	0.2	100
Kralj E et al. [51]	A	2012	Nilotinib, imatinib, dasatinib	^{13}C-D3-nilotinib	50–5000	50[b]	<10.3	LLE	0.8	200
Couchman L et al. [54]	A	2012	Nilotinib, other 7 TKIs	$^{13}C_2$–$^{15}N_2$-nilotinib	100–5000	? < 10[d]	<8.72	LLE	0.3	50
Lankheet NA et al. [56]	A	2013	Nilotinib, other 7 TKIs	D3-nilotinib	20–10,000	20[b]	<4.8	LLE	0.3	50
van Erp NP et al. [57]	A	2013	Nilotinib, other 5 TKIs	$^{13}C_3$–^{15}N-nilotinib	100–4000	100[d]	<5.4	LLE	0.1	50
Andriamanana I et al. [58]	A	2013	Nilotinib, other 8 TKIs	D8-imatinib	50–3500	50[b]	<7.7	LLE	0.2	50

A; LC–MS/MS, B; LC–MS, IS; internal standard, LOQ; limits of quantitation, CV; coefficient of variations (included intra-day and inter-day), TKIs; tyrosine kinase inhibitors, LLE; liquid–liquid extraction, SPE: solid-phase extraction, Conc. rate; concentration rate = plasma sample volume*recovery/pre-injecting sample volume.

[a] Global standard.
[b] 20%CV value.
[c] Signal-to-noise ratio of 10.
[d] Signal-to-noise ratio of 5.

Table 6
HPLC–UV methods for the quantitation of dasatinib in human plasma.

Reference	Year	Analyte(s)	IS	UV	Calibration range (ng/mL)	LOQ (ng/mL)	CV (%)	Extraction	Conc. rate	Sample volume (μL)
Miura M et al. [26]	2011	Dasatinib	Imatinib	250	5–200	5[a]	<18.5	SPE	3.7	100
Pirro E et al. [28]	2011	Dasatinib, Imatinib	Nilotinib	267	5–10,000	100[a]	<18.47	LLE–SPE	1.0	500

IS; internal standard, UV; ultra-violet, LOQ; limits of quantitation, CV; coefficient of variations (included intra-day and inter-day), LLE; liquid–liquid extraction, SPE: solid-phase extraction, Conc. rate; concentration rate = plasma sample volume * recovery/pre-injecting sample volume.
[a] 20%CV value.

C_0 increased the risk of cumulative incidences of pleural effusion [4,5,76–78]. In contrast to imatinib and nilotinib, the main purpose of TDM for dasatinib is the avoidance of side effects [5]. The administration of dasatinib 100 mg once daily is the standard dosage to obtain sufficient efficacy with reduced side effects [76,79,80]. By carrying out TDM with a target concentration of 3 nM (1.5 ng/mL) after beginning with an initial dasatinib daily dose of 100 mg, the mean maintenance daily dose of dasatinib was reported by Rousselot et al. to be 57 mg [78].

In the OPTIM dasatinib trial, the dasatinib C_0 in 289 CML patients ranged from 0.1 nM (0.05 ng/mL) to 18.7 nM (9.5 ng/mL). In our routine quantification of dasatinib in CML outpatients, the dasatinib C_0 ranged from 0.1 ng/mL to 11 ng/mL (mean 2.04 ng/mL). In the phase III studies [76,77], the dasatinib C_0 was measured using the LC–MS/MS method developed by Dai et al. with a LOQ of 1.0 ng/mL [81]. It has been reported that the analysis dataset in the former Phase III study contained 4044 measurements of dasatinib plasma concentrations from 399 patients; however, about 4.7% of the available dasatinib concentration measurements were below the LOQ of 1.0 ng/mL and were excluded from the analysis dataset [76]. Therefore, a LOQ of less than 1.0 ng/mL for quantification of dasatinib in plasma is necessary [5], and the HPLC–UV method of Miura et al. also could not be used to quantify a dasatinib C_0 less than 5.0 ng/mL [26].

On the other hand, the steady-state mean maximum plasma concentration of dasatinib (C_{max}) in East Asian and non-East Asian patients taking 100 mg once daily were 76.9 ng/mL and 57.4 ng/mL, respectively [77]. The dasatinib plasma concentration at 2 h (C_2) or C_{max} might be set above 50 ng/mL to obtain sufficient efficacy and to avoid low exposure of dasatinib because of the risk of developing BCR-ABL point mutations [5,82–84]. Thus, the therapeutic target for dasatinib is to maintain a higher C_{max} or C_2 (above 50 ng/mL) and a lower C_0 (less than 1.5–2.5 ng/mL) after administration of dasatinib 100 mg once daily [5]. Consequently, plasma concentrations of dasatinib need to be in the range between 0.1 ng/mL and 200 ng/mL (considering a higher C_{max}).

LC–MS/MS and LC–MS methods for the quantification of dasatinib in human plasma reported to date are shown in Table 7 [30,45,47,50,51,54,56–58,81,85,86]. Three analytical methods have a LOQ of less than 1.0 ng/mL (around 0.1 ng/mL) for dasatinib [30,50,86]. Currently, the LC–MS/MS method of MASIS Inc is routinely used for the observational study of *de novo* chronic myeloid leukemia patients in the chronic phase in Japan and for the observational study of chronic myeloid leukemia patients in the chronic phase with resistance or intolerance to preceding TKI therapy in Japan (New TARGET, The Japanese Society of Hematology) [86]. In contrast to imatinib and nilotinib, the target dasatinib C_0 is remarkably low. Therefore, for determination of dasatinib in

Table 7
LC–MS/MS (LC–MS) methods for the quantitation of dasatinib in human plasma.

Reference		Year	Analyte(s)	IS	Calibration range (ng/mL)	LOQ (ng/mL)	CV (%)	Extraction	Conc. rate	Sample volume (μL)
Dai G et al. [81]	A	2008	Dasatinib	$^{13}C_4$–$^{15}N_2$-dasatinib	1–1000	1[No data]	<8.2	SPE	–	No data
De Francia S et al. [45]	B	2009	Dasatinib, nilotinib, imatinib	Quinoxaline	62.5–8000	62.5[a]	<11.4	LLE	0.3	250
Haouala A et al. [47]	A	2009	Dasatinib, other 5 TKIs	D8-imatinib	1–200	1[a]	<9.4	LLE	0.17	100
Bouchet S et al. [50]	A	2011	Dasatinib, other 8 TKIs	Dasatinib-M+6	0.1–160	0.1[a]	<19.5	SPE	2.7	300
Kralj E et al. [51]	A	2012	Dasatinib, imatinib, nilotinib	D8-dasatinib	2.5–250	2.5[a]	<9.9	LLE	0.7	200
Couchman L et al. [54]	A	2012	Dasatinib, other 7 TKIs	D10-sunitinib	1–150	? < 1[b]	<11.72	LLE	0.3	50
Furlong MT et al. [85]	A	2012	Dasatinib, M4, M6	$^{13}C_4$–$^{15}N_2$-dasatinib	1–1000	1[a]	<10.7	SPE	–	No data
Lankheet NA et al. [56]	A	2013	Dasatinib, other 7 TKIs	D3-dasatinib	5–2500	5[a]	<11.3	LLE	0.3	50
van Erp NP et al. [57]	A	2013	Dasatinib, other 5 TKIs	D3-dasatinib	5–400	5[b]	<17.7	LLE	0.1	50
Andriamanana I et al. [58]	A	2013	Dasatinib, other 8 TKIs	D8-imatinib	2–250	2[a]	<11.6	LLE	0.2	50
Birch M et al. [30]	A	2013	Dasatinib, imatinib, N-DI	D8-imatinib	1–200	0.13[b]	<13	LLE	0.2	50
MASIS Inc [86].	A	2013	Dasatinib	D8-dasatinib	0.1–100	0.1[c]	<10	LLE	1.0	200

A; LC–MS/MS, B; LC–MS, IS; internal standard, LOQ; limits of quantitation, CV; coefficient of variations (included intra-day and inter-day), N-DI; N-desmethyl imatinib, TKIs; tyrosine kinase inhibitors, LLE; liquid–liquid extraction, SPE: solid-phase extraction, Conc. rate; concentration rate = plasma sample volume*recovery/pre-injecting sample volume.
[a] 20%CV value.
[b] Signal-to-noise ratio of 5.
[c] Signal-to-noise ratio of 10.

Table 8
A recommended analytical condition for the quantitation of imatinib, nilotinib and dasatinib in human plasma.

Analyte	Sampling time	LOQ (ng/mL)	Required calibration range (ng/mL)	Sample volume (μL)
Imatinib	Trough (just prior taking)	10	10–5000	100–200
Nilotinib	Trough (just prior taking)	10	10–4000	
Dasatinib	2 h after taking Trough (just prior taking)	0.1	0.1–200	

LOQ; limits of quantitation.

human plasma, LC–MS/MS methods with high sensitivity are superior to HPLC–UV methods, because in the low area of plasma concentration, this highly selective technique results in minimal interference from endogenous impurities. The LC–MS/MS method developed by Bouchet et al. requires a comparatively large sample volume of 300 μL; however, the concentrations of imatinib, nilotinib and dasatinib could be assayed simultaneously and at higher sensitivity with a LOQ of 10 ng/mL, 10 ng/mL and 0.1 ng/mL, respectively, in the best calibration range [50]. At present, the LC–MS/MS method of Bouchet et al. might be the most appropriate method for the simultaneous quantitation of TKIs in patients.

5. Conclusions

Analytical methods using HPLC–UV or LC–MS/MS have been reported for the quantification of imatinib, nilotinib, and dasatinib in biological fluids. LC–MS/MS methods can be used to quantify these TKIs and their metabolites simultaneously, and they have high sensitivity and selectivity in the low plasma concentration area of less than 1 ng/mL. For quantification of imatinib, nilotinib, and dasatinib, a LOQ of less than 10 ng/mL, 10 ng/mL, and 0.1 ng/mL, respectively, in the clinical setting is necessary (Table 8). Because a simpler and more cost-efficient methodology is desired for clinical monitoring, imatinib and nilotinib plasma concentrations could be assayed by HPLC–UV methods after comparison with the results obtained from the standard LC–MS/MS method. However, in the quantification of dasatinib, the highly sensitive LC–MS/MS method is superior to HPLC–UV methods. Analytical methods with high precision are needed for individualized treatment through adapting the dose of oral anticancer drugs.

Conflict of interest

MM has no conflicts of interest.

NT received research funding and honoraria for lectures from Novartis Pharmaceuticals and Bristol-Myers Squibb.

Disclosure

The authors have no relevant relationships to disclose.

Acknowledgments

This work was supported by grant numbers 26460188, 26461414, and 23590168 from the Japan Society for the Promotion of Science, Tokyo, Japan.

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