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.