Journal of Chromatography B, 1023-1024 (2016) 24–29

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Liquid chromatography–tandem mass spectrometric assay for ponatinib and *N*-desmethyl ponatinib in mouse plasma

Rolf W. Sparidans[a,b,*], Anita Kort[c], Alfred H. Schinkel[c], Jan H.M. Schellens[a,d], Jos H. Beijnen[a,d,e]

[a] *Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Division of Pharmacoepidemiology & Clinical Pharmacology, Universiteitsweg 99, 3584CG Utrecht, The Netherlands*
[b] *Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Division of Chemical Biology & Drug Discovery, Universiteitsweg 99, 3584CG Utrecht, The Netherlands*
[c] *The Netherlands Cancer Institute, Division of Molecular Oncology, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands*
[d] *The Netherlands Cancer Institute, Department of Clinical Pharmacology, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands*
[e] *MC Slotervaart, Department of Pharmacy & Pharmacology, Louwesweg 6, 1066 EC Amsterdam, The Netherlands*

ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 25 April 2016
Accepted 30 April 2016
Available online 2 May 2016

Keywords:
Ponatinib
N-desmethyl ponatinib
LC–MS/MS
Mouse plasma
Liquid-liquid extraction

ABSTRACT

Ponatinib is a multi-targeted third generation BCR–ABL1 tyrosine-kinase inhibitor approved for specific types of leukemia. A bioanalytical assay for this drug and its *N*-desmethyl metabolite in mouse plasma was developed and validated using liquid chromatography-tandem mass spectrometric (LC–MS/MS) with liquid-liquid extraction as sample pre-treatment procedure.

After extraction with *tert*-butyl methyl ether of both analytes with their isotopically labeled internal standards and evaporation and reconstitution of the extract, compounds were separated by reversed-phase liquid chromatography under alkaline conditions. After electrospray ionization, both compounds were quantified in the selected reaction monitoring mode of a triple quadrupole mass spectrometer.

The linear assay was validated in the ranges 5–5000 ng/ml for ponatinib and 1–1000 ng/ml for *N*-desmethyl ponatinib. Within-run ($n = 18$) and between-run (3 runs; $n = 18$) precisions were 10% and 12% at the lower limit of quantification for the metabolite, all other precisions were ≤8% for the metabolite and ≤6% for ponatinib. Accuracies were between 92 and 108% for both compounds in the whole calibration range. The drug was sufficiently stable under most relevant analytical conditions, only ponatinib showed more than 15% hydrolytic degradation after storage for 6 h and longer at ambient temperature in mouse plasma. Finally, the assay was successfully applied to determine plasma drug levels and study pharmacokinetics after oral administration of ponatinib to female FVB mice.

1. Introduction

Ponatinib (Iclusig®, AP24524, Fig. 1A) is a multi-targeted third generation BCR–ABL1 tyrosine-kinase inhibitor, being the first in its class. In contrast to the first (imatinib) and second (nilotinib, dasatinib, bosutinib) generation drugs it is also active for the BCR-ABL1 T315I and other mutations [1,2]. Therefore, ponatinib can be highly active in Philadelphia chromosome-positive (Ph+) leukemia [3,4]. Mainly because of serious cardiovascular risks [5], vascular occlusions and heart failure, but also hepatotoxicity, its use remains limited to second-generation drug-resistant and (T351I) mutated patients with Ph+ acute lymphoblastic leukemia (ALL) [6] or as third-line therapy in Chronic Myeloid leukemia (CML) [7].

Bioavailability of ponatinib is unknown, plasma protein binding is high (99%) and plasma half-life ($t_{1/2}$) is *ca.* 24 h in human [8]. The drug is mainly eliminated via feces after Phase I and II

Abbreviations: ALL, acute lymphoblastic leukemia; BCR-ABL, breakpoint cluster region-Abelson murine leukemia viral oncogene homolog; CML, chronic myeloid leukemia; CYP3A4, cytochrome P450 3A4; DMP, *N*-desmethyl ponatinib; ESI, electrospray Ionization; FVB, friend leukemia virus strain B; IS, internal standard; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LLOQ, lower limit of quantification; Ph+, philadelphia chromosome-positive; QC, quality control; SRM, selected reaction monitoring; tBME, *tertiary*-butyl methyl ether; TKI, tyrosine kinase inhibitor.

* Corresponding author at: Utrecht University, Faculty of Science, Department of Pharmaceutical Sciences, Division of Pharmacoepidemiology & Clinical Pharmacology, Universiteitsweg 99, 3584CG Utrecht, The Netherlands.
E-mail address: R.W.Sparidans@uu.nl (R.W. Sparidans).

http://dx.doi.org/10.1016/j.jchromb.2016.04.049
1570-0232/

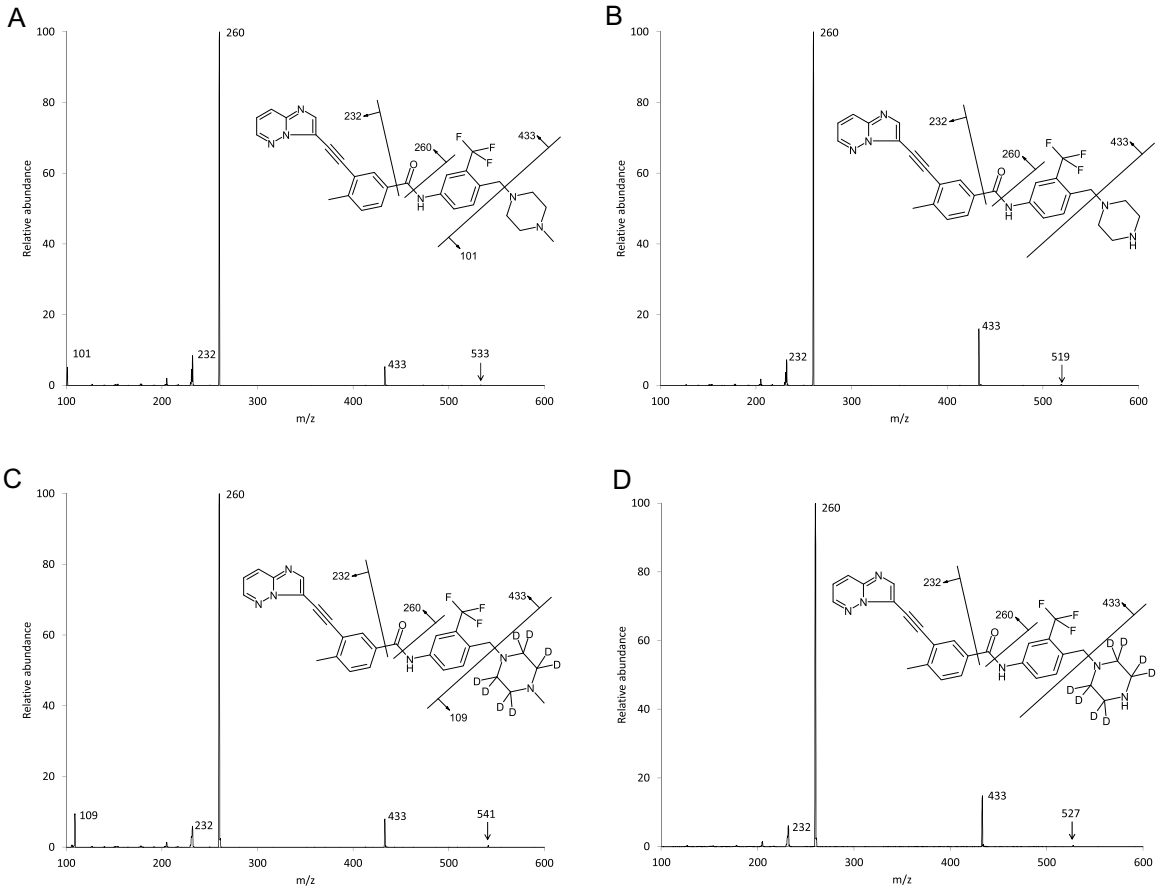

Fig. 1. Chemical structures and product spectra, formed by collision-induced dissociation (−29 V) of the protonated molecules of (A) ponatinib(3-(imidazo[1,2-*b*]pyridazin-3ylethynyl)-4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide); *m/z* 533.2, (B) DMP; *m/z* 519.2, (C) ponatinib-d_8; *m/z* 541.2 and (D) DMP-d_8; *m/z* 527.2. Dissociation pathways of the fragmentation reactions have been proposed.

biotransformation. Extensive metabolic studies of ponatinib have not yet fully been reported but many metabolites could be found in excreta [8] and the main circulating metabolite was found to be the inactive carboxylic acid AP24600 formed by esterase- and/or amidase-mediated hydrolysis [8,9]. An active *N*-desmethyl (DMP, AP24567, Fig. 1B) and *N*−oxide metabolite (AP24734) were additionally formed by Cytochrome P450 (mainly CYP3A4) biotransformation and found in plasma together with a hydroxy metabolite [9]. *N*-oxidation and hydroxylation both take place at an unknown position on the methyl piperazinyl group [8].

Recently, a micellar enhanced spectrofluorometric assay for ponatinib in human plasma and urine was reported [10] that was able to quantify clinical levels of the drug. This method, however, uses a large sample volume (1 ml) and is not able to distinguish the parent compound from the active DMP metabolite and other metabolites. LC−MS/MS will be a more sensitive and selective technique and therefore more suited for mouse studies with low sample volumes. So far, it has been used in several human pharmacokinetic studies to quantify ponatinib in plasma alone [11], with the active DMP metabolite [12], and with the inactive carboxylic acid metabolite [9]. All LC–MS/MS assays use solid-phase extraction, in a 96-wells format with Oasis MCX [9] or unspecified Isolute [11,12] columns, and positive electrospray ionization but reported validation results [8] of the assays were limited. All assays obtained a lower limit of quantification (LLOQ) at 0.5 ng/ml ponatinib using 75 [9] or 150 [11,12] μl of human plasma. Stable isotopically labeled ponatinib (ponatinib-d_3) was used in two of the assays [9,11,12]. For mouse studies we therefore present a new completely validated LC–MS/MS method dedicated to smaller sample volumes with inclusion of the active metabolite.

2. Experimental

2.1. Chemicals

Ponatinib (>98%) was supplied by Sequoia Research Products (Pangbourne, United Kingdom), DMP (97.0%), ponatinib-d_8 (99.5%, isotopic purity >99%, Fig. 1C) and DMP-d_8 (96.8%, isotopic purity >99%, Fig. 1D) were obtained from Alsachim (Illkirch Graffenstaden, France). Water (LC–MS grade) and methanol (HPLC grade) were obtained from Biosolve (Valkenswaard, The Netherlands). Water not used as eluent was home purified by reversed osmosis on a multi-laboratory scale. Ammonium hydroxide (ACS reagent grade) originated from Sigma-Aldrich (Steinheim, Germany) and *tert*-butyl methyl ether (tBME, Lichrosolve quality) from Merck (Darmstadt, Germany). Pooled mouse lithium heparin plasma was supplied by Seralab Laboratories International (Haywards Heath, UK).

2.2. Equipment

The LC–MS/MS equipment consisted of an Accela pump and autoinjector and a TSQ Quantum Ultra triple quadrupole mass spectrometer with heated electrospray ionization (HESI), all supplied by Thermo Fisher Scientific, San Jose, CA, USA. Data were recorded and the system was controlled using the Thermo Fisher Xcalibur software (version 2.07).