

PONATINIB IN PLASMA BY LC/MS

Code LC99010

This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD).

The declaration of conformity (CE) is available upon request.



EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com

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INTRODUCTION

Ponatinib (Iclusig®), has been approved for the treatment of patients with Chronic Myeloid Leukemia (CSF) in the chronic, accelerated or blastic phase, resistant or intolerant to dasatinib or nilotinib and for whom subsequent treatment with imatinib is not clinically appropriate, or in whom the T315I mutation has been identified.

Ponatinib has been approved at a dose of 45 mg/day, with associated plasma levels of steady-state drug ≥ 40 nM, a concentration required to prevent the growth of other mutated BCR-ABL clones (1).

Unfortunately, the clinical use of ponatinib is limited by the possible occurrence of occlusive vascular events (thrombosis and arterial and venous occlusions, including fatal myocardial infarction, stroke, stenosis of the large arterial vessels of the brain, severe peripheral vasculopathy) (2). The mechanism or mechanisms underlying this cardiovascular toxicity are still not entirely clear. However, a close correlation between the dose of ponatinib and the risk of cardiovascular events has been considered, so much so as to suggest a reduction in the dosage of medication to mitigate the risk of cardiovascular events. In particular, it has been demonstrated that for every 15 mg of reduced ponatinib there is a 33% reduction in the risk of arterial thrombosis (3).

From a pharmacokinetic point of view, such a clear relationship between dose and cardiovascular risk seems to be based on prolonged and unnecessary plasma exposure to plasma levels of ponatinib ≥ 40 nM. Lower doses, such as 30 or 15 mg/day, could still result in significant plasma exposure (4), thus maintaining antileukemic activity and reducing the risk of cardiovascular events (5).

For CML patients the data sheet has been modified to allow clinicians to reduce the dose of ponatinib from 45 to 15 mg/day once cytogenetic response has been obtained (2). The decision is left to the discretion of the clinicians and to the evaluation of the patient's characteristics and individual risk factors.

A recently published case report demonstrated the efficacy of low-dose ponatinib in a patient with Acute Philadelphia positive Lymphoblastic Leukemia. The authors demonstrated that during the reduction of ponatinib doses the plasma concentrations of the drug were always ≥ 40 nM (6).

The Eureka Lab Division diagnostic KIT allows to constantly monitor the plasma concentrations of ponatinib during the therapy of each individual patient; therefore, it represents for clinicians an important support tool, fast and cheap, able to guarantee them the possibility of avoiding excessive plasma exposure to ponatinib that could cause thrombotic events.

References

1. O'hare O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell*. 2009; 16:401-12.
2. Moslehi JJ, Deininger M Tyrosine Kinase Inhibitor-Associated Cardiovascular Toxicity in Chronic Myeloid Leukemia. *J Clin Oncol*. 2015 Dec 10;33(35):4210-8.
3. Dorer DJ, Knickerbocker RK, Baccarani M, Cortes JE, Hochhaus A, Talpaz M et al. Impact of dose intensity of ponatinib on selected adverse events: Multivariate analyses from a pooled population of clinical trial patients. *Leuk. Res*. 2016; 48:84-91.
4. Cortes JE, Kantarjian H, Shah NP, Bixby D, Mauro MJ, Flinn I et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. *N Engl J Med*. 2012; 29:2075-88.
5. Breccia M, Pregno P, Spallarossa P, Arboscello E, Ciceri F, Giorgi M et al. Identification, prevention and management of cardiovascular risk in chronic myeloid leukaemia patients candidate to ponatinib: an expert opinion. *Ann Hematol*. 2017; 96:549-558.
6. Marchesi F, Salvatorelli E, Renzi D, Mengarelli A, Minotti G and Menna P. Efficacy and safety of low dose ponatinib in a case of Ph-positive acute lymphoblastic leukaemia. *British Journal of Haematology* 2019;187, e1–e32.

TECHNICAL FEATURES

RECOVERY:	87,5 %		
SENSITIVITY (LLOD):	0,17 ng/ml		
MINIMUM CONCENTRATION ANALYZABLE (LLOQ):	0,5 ng/ml		
LINEARITY:	0,5 – 1.000 ng/ml		
<u>Accuracy intra serie (relative error %) :</u>	Ci	Cs	
	5 ng/ml	50 ng/ml	
	6,16%	3,26%	
<u>Accuracy inter serie (relative error %) :</u>	Ci	Cs	
	5 ng/ml	50 ng/ml	
	9,96%	4,98%	
<u>Reproducibility intra serie (coefficient of variation %):</u>	C LLOQ	Cm	CUP
	0,5 ng/ml	25 ng/ml	100 ng/ml
	4,85%	1,82%	2,78%
<u>Reproducibility inter serie (coefficient of variation %):</u>	C LLOQ	Cm	CUP
	0,5 ng/ml	25 ng/ml	100 ng/ml
	7,21%	7,09%	7,04%
<u>Coefficient of Correlation R2 + Dev Std:</u>	0,9981 ± 0,0014		

COMPONENTS OF THE KIT (100 TESTS)

Reagent A – Deproteinization Solution + Internal Standard **1 x 40 ml** **Store at -20 °C**

Calibrator in urine – Level 0	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Calibrator in urine – Level 1	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Calibrator in urine – Level 2	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Calibrator in urine – Level 3	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Calibrator in urine – Level 4	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Calibrator in urine – Level 5	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Calibrator in urine – Level 6	1 x 1 ml	Code LC99016 (Packed separately – see technical sheet)
Reagente M1 – Fase Mobile M1	1 x 500 ml	
Reagente M2 – Fase Mobile M2	2 x 500 ml	

All the reagents are ready to use and stable 3 years at 2-8 °C,
except the Reagent A that must be stored at -20 °C.

As regards the preservation method of the Lyophilized Calibration Standard, it is described in the dedicated technical sheet.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC99016	Calibrator in plasma for Ponatinib	7 x 2 x 1 ml
LC99019	Control in plasma for Ponatinib - Levels 1, 2 e 3	3 x 5 x 1 ml
SK99010	Starter kit for Ponatinib in plasma	1 Pc
For UHPLC systems		
S959757902	Zorbax RRHD C18 (50 x 2,1 mm, 1,8 um) Analytical Column	1 Pc
For HPLC systems		
Z959793902	Zorbax Eclipse C18 (100 x 2,1 mm, 3,5 um) Analytical Column	1 Pc

MINIMUM INSTRUMENTAL EQUIPMENT REQUIRED

LC-MS/MS Triple Quadrupole with binary pump
MRM mode, ESI positive

OPTIONAL EQUIPMENT

Autosampler
Operational Computer

WHOLE BLOOD COLLECTION PROCEDURE

Take 3 ml of venous blood in a tube with EDTA as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Separate the plasma in a glass tube and then store at -20°C. Stable 1 month.

ANALYTICAL PROCEDURE

STEP 1

Dispense in eppendorf:

- 100 µl of **Calibrator (C0-C6)/Controls/Samples**
- 400 µl of **Reagent A – Deproteinization Solution + Internal Standard**

Vortex for 10 sec.

Centrifuge at 14.000 rpm for 10 minutes

STEP 2

Transfer the supernatant in a vial.

N.B.: at this step, the sample is stable 1 day at 2-8 °C

INJECTION

- Inject 2-10 µl of supernatant in HPLC.

PONATINIB IN PLASMA by LC/MS - Warnings

COLUMN CONDITIONING

Install a new analytical column Zorbax Eclipse C18 (100 x 2,1 mm, 3,5 μ m) or Zorbax RRHD C18 (50 x 2,1 mm, 1,8 μ m), thermostated at 40 °C. Disconnect the detector and flux a una solution of Mobile Phase M2 : Mobile Phase M1 (90 : 10 v/v) set flow at 350 μ l / minute for 20 minutes. Condition further fluxing a solution of Mobile Phase M2 : Mobile Phase M1 (90 : 10 v/v) for 15 minutes. **Don't recycle the washing solutions.**

It is NOT possible to make analysis at recycling phase.

If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Wash with a solution of Mobile Phase M2 : Mobile Phase M1 (90 : 10 v/v) at flow of 350 μ l / minute for 20 minutes. The column must be stored in this solution.

INJECTION NEEDLE WASHING

Wash the injection needle with a solution of Methanol : H₂O (85 : 15 v/v).

PARAMETERS SET ON WATERS XEVO TQS MICRO

Capillary Voltage (kV)	1,0
Desolvation Gas (L/hour)	1000
Desolvation Temperature (°C)	550
Source Temperature(°C)	150

FRAGMENTATIONS (optimized on TRIPLE QUADRUPOLE WATERS XEVO TQS MICRO)

MOLECULE	PARENT ION	DAUGHTER ION	COLLISION ENERGY
PONATINIB-1	533.4	232.1	46
PONATINIB-1	533.4	260.2	28
PONATINIB-D8	541.5	260.2	36

GRADIENT SET

Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0	90	10	350
0.5	70	30	350
1.0	35	65	350
1.5	10	90	350
5.0	10	90	350
5.1	90	10	350
10.0	90	10	350

PONATINIB IN PLASMA BY LC/MS

(Reference Chromatograms)

Fig. 1 :	Calibrator in plasma		
	R.T. 2.36	Ponatinib	100 ng/ml
	R.T. 2.36	Ponatinib-D8	

Fig. 2 :	Calibrator in plasma		
	R.T. 2.36	Ponatinib	10 ng/ml
	R.T. 2.36	Ponatinib-D8	

