A Simple and Sensitive HPLC Method for the Detection and Quantitation Of STI-571 And Its Main Metabolite N-Desmethyl-STI

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An isocratic online enrichment HPLC-assay was developed allowing for the simple and fast separation and quantitation of STI-571 and its main metabolite N-Desmethyl-STI in plasma, urine, cerebrospinal fluid, culture media and cell preparations in various concentrations using UV-detection at 260 nm. The analytical procedure consists of an online concentration of STI-571 and N-Desmethyl-STI in the HPLC system followed by the elution on a ZirChrom®- PBD analytical column.

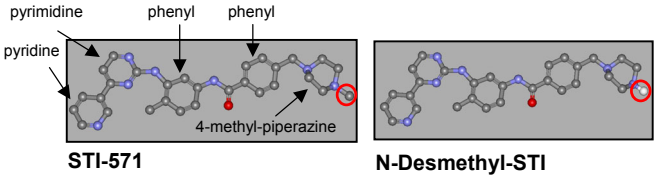

Figure 1. Structures of STI-571 and N-Desmethyl-STI

Introduction

STI-571 (Imatinib mesylate, Glivec™), a 4-[(4-methyl-1-piperazinyl)methyl]-N-[4- [1–3] methyl - 3 - [[4 - (3 - pyridinyl) - 2 - pyrimidinyl]amino]- phenyl] benzamide methane-sulfonate derivative, acts as an inhibitor of the abl tyrosine kinase, platelet derived growth factor receptor (PDGFR), stem cell factor receptor (c-kit, steel factor receptor, CD117) and ARG tyrosine kinases. The specific blockade of the bcr-abl oncoprotein has been associated with significant antileukemic activity in patients with chronic-myeloid-leukemia (CML) and Philadelphia-positive-acute-lymphatic-leukemia (Ph+ALL).

This analytical method was developed by Universitätsklinikum Carl Gustav Carus an der Technischen Universität (Dresden, Germany) to perform pharmacokinetic measurements of STI-571 and N-Desmethyl-STI in patient samples (plasma, urine, cerebrospinal fluid) and for kinetic measurements of intracellular STI-571 and N-Desmethyl-STI following in-vitro incubation [1]. This method utilizes UV detection but may also be adapted to electrochemical detection to enable lower detection limits.

Experimental

A mixture of STI-571 and N-Desmethyl-STI was separated at room temperature using a ZirChrom®-PBD guard column, a ZirChrom®-PBD analytical column and UV detection. This analytical method includes an online-enrichment system incorporating another ZirChrom®-PBD guard column and an electric motor driven switching valve (refer to [1] for schematic valve switching graph). The separation conditions were as follows:

Enrichment

Guard Column:	ZirChrom®-PBD, 10 mm x 4.6 mm i.d. Guard Insert (Part Number: ZR03-G40; set of 3); Guard Insert Holder (Part Number 850-00)
Mobile Phase:	45/35/20 (v/v) 0.1 M dibasic potassium phosphate/water/methanol
Temperature:	Uncontrolled

Analytical

Guard Column:	ZirChrom®-PBD, 10 mm x 4.6 mm i.d. Guard Insert (Part Number: ZR03-G40; set of 3); Guard Insert Holder (Part Number 850-00)
Column:	ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number ZR03-0546)
Mobile Phase:	60/40 (v/v) 10 mM dibasic potassium phosphate, 90 mM monobasic potassium phosphate/methanol
Temperature:	Uncontrolled
Flow Rate:	0.4 ml/min.
Injection:	50 µl
Detection:	UV at 260 nm

Time of analysis is 40 minutes including the enrichment time of 5 minutes. The UV detection limit is 10 ng/ml in plasma, CSF (cerebrospinal fluid), culture medium (RPMI) and 25 ng/ml in urine for both STI-571 and N-Desmethyl-STI.

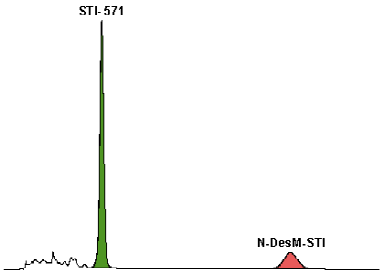

Figure 2: Chromatogram of patient plasma under STI-571 treatment with 5308 ng/ml STI-571 (RT ≈ 10.0 min.) and 988 ng/ml N-Desmethyl-STI (RT ≈ 30.0 min.).

This method can be tailored to your specific application needs. ZirChrom method developers can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details.

Acknowledgements

Universitätsklinikum Carl Gustav Carus an der Technischen Universität (Dresden, Germany)

References

[1] E. Schleyer, S. Pursche, C.H. Köhne, U. Schuler, U. Renner, H. Gschaidmeier, J. Freiberg-Richter, T. Leopold, A. Jenke, M. Bonin, T. Bergemann, P. le Coutre, M. Gruner, M. Bornhäuser, O.G. Ottmann, G. Ehninger, J. Chromatogr. B, 799, (2004) 23-36.

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