https://ijrps.com

ISSN: 0975-7538
Research Article

Development and validation of stability indicating liquid chromatography tandem mass spectrometry method for the determination of Dasatinib in bulk

Babu B*[1], Meyyanathan SN[1], Gowramma B[2], Chandrasekar MJN[2], Narenderan ST[1]

[1]Department of Pharmaceutical Analysis, JSS College of Pharmacy, Ootacamund, Jagadguru Sri Shivarathreeshwara University, Mysore-643001, India
[2]Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Ootacamund, Jagadguru Sri Shivarathreeshwara University, Mysore-643001, India

ABSTRACT

Dasatinib is a medication used primarily to treat chronic myelogenous leukemia and selected cases of acute lymphoblastic leukemia. In the present study a validated stability-indicating LC-MS/MS method was developed for the estimation of Dasatinib and its degradants on Hypersil ODS (150mm x 4.6 mm i.d., 5µ) column using Methanol: 10mM Ammonium Formate as the mobile phase in a isocratic elution at a flow rate of 1.0 mL/min. The method was validated for accuracy, precision and linearity as per the ICH guidelines. The forced degradation studies were carried out for Dasatinib under acidic, basic, thermal, reduction and oxidation conditions, among which oxidative degradation and basic degradation had above 90 % and 30 % degradation. The degradation products were characterized by MS-MS. The developed method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug. The recoveries of Dasatinib were found to be within the range.

Keywords: Dasatinib; LC-MS/MS; force degradation; ICH.

INTRODUCTION

Dasatinib is a medication given orally for treating chronic myeloid leukemia and acute lymphoblastic leukemia. It is classified as a kinase inhibitor, which prevent the growth of tumors by reducing the action of proteins that control cell division, growth, and survival. The reduction of these proteins, growth and survival of cancer cells are inhibited. The chemical name for Dasatinib is N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-yl]amino]-1,3-thiazole-5-carboxamide (Figure 1). The molecular formula is $C_{22}H_{26}ClN_7O_2S$, where anhydrous free base has a molecular weight of 488.01. The monohydrate corresponds to a formula weight of 506.02.

Literature survey revealed that few bio-analytical methods are developed for estimation ofDasatinib in formulation (Antonio D'Avolio, 2012; Eva Kralj, 2012; Haouala. A, Zanolari, 2009; Hassan Karimi-Maleh, 2016; Irina Andriamanana, 2013; MarekDziadosz, 2012; Michael T. Furlong, 2012; Mohammed G. Kassem, 2013; Silvia De Francia, 2009;StéphaneBouchet, 2011) and stability indicating method of Dasatinibwas reported HPTLC (Mhaske. D. V, 2007). The present paper presents an effective separation, selective and high sensitive stability indicating method for the determination of Dasatinib and its degradants.

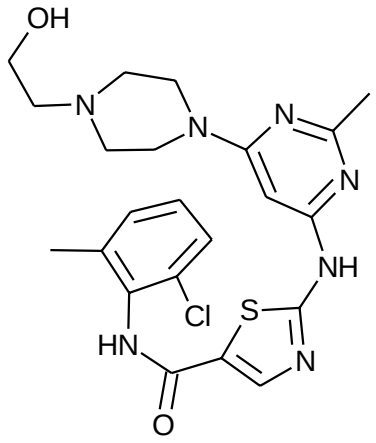

Figure 1: Structure of Dasatinib

MATERIALS AND METHODS

Equipment

High Performance Liquid Chromatography **(**Shimadzu gradient HPLC system**)** equipped with a solvent delivery system **(**Model-LC-10 AT-VP**)**, Rheodyne injector **(**Model-7725i with 20µl loop**)**. The data were recorded using Class VP data station software. Hypersil ODS (150mm x 4.6 mm i.d., 5µ) column was used for method development and validation at ambient temperature **(**25°C**).** In this current method the separation was

* Corresponding Author
Email: thammababu@gmail.com
Contact: +91-8667636779
Received on: 11.09.2017
Revised on: 14.11.2017
Accepted on: 23.11.2017

achieved using 1ml/minflow rate, detection at 290nm with an injection volume of 20µl. LC-MS/MS (Shimatzu 8030, Tokyo japan) equipped with electrospray ionization (ESI) interface, LC-20AD pump, SPD-M20 PDA detector, CTO-20AC column oven, CBM-20 alite controller and SIL-20AC auto sampler using Lab solution data station.

Forced degradation

Stress studies were carried out according to ICH guidelines Q1A(R2). The API was subjected to forced degradation under acidic, basic and neutral conditions by refluxing in 1 N HCl, 1N NaOH at room temperature and oxidative stress studies was carried out using 30% H_2O_2 at room temperature for 2, 4, 6, 8, 12 and 24hr, respectively.

at 350°C, CID gas maintained at 230Kpa, N_2 was used as nebulizing and drying gas flow was set at 3 L/min and 15 L/min respectively.

Validation parameters

A stock solution containing 1 mg/ mL was diluted to produce a concentration range of 10–200 µg/mL to establish linearity and range. The analysis was carried out in triplicate by injecting. The intra- and inter-day precision were established by analyzing for QC concentration of 50, 100 and 200 µg/mL of drug solution (Table 1), respectively. Accuracy of the method was determined by analyzing QC concentrations 50, 100 and 200 µg/mL spiked with stressed sample in triplicate and then determining the percent recovery (Table 1). The signal to noise ratios were 3:1 and 10:1 for determining limit of

Table 1: Recovery, Precision studies for the Dasatinib

Actual Conc. (µg/ml)	Recovered Conc (µg/ml) ±SD; RSD% (n=6)	% Recovery	Intraday calculated concentration (µg/mL) ± S.D.; RSD % (n=5)	Interday calculated concentration(µg/mL) ± S.D.; RSD % (n=5)
50	49.92 ± 0.01, 0.021	101.58	50.00 ± 0.11, 0.222	50.00 ± 0.19, 0.375
100	99.81 ± 0.17, 0.342	101.61	100.00 ± 0.25, 0.250	100.00 ± 0.21, 0.231
200	199.32 ± 0.15, 0.152	98.32	199.64 ± 0.27, 0.314	199.99 ± 0.26, 0.129

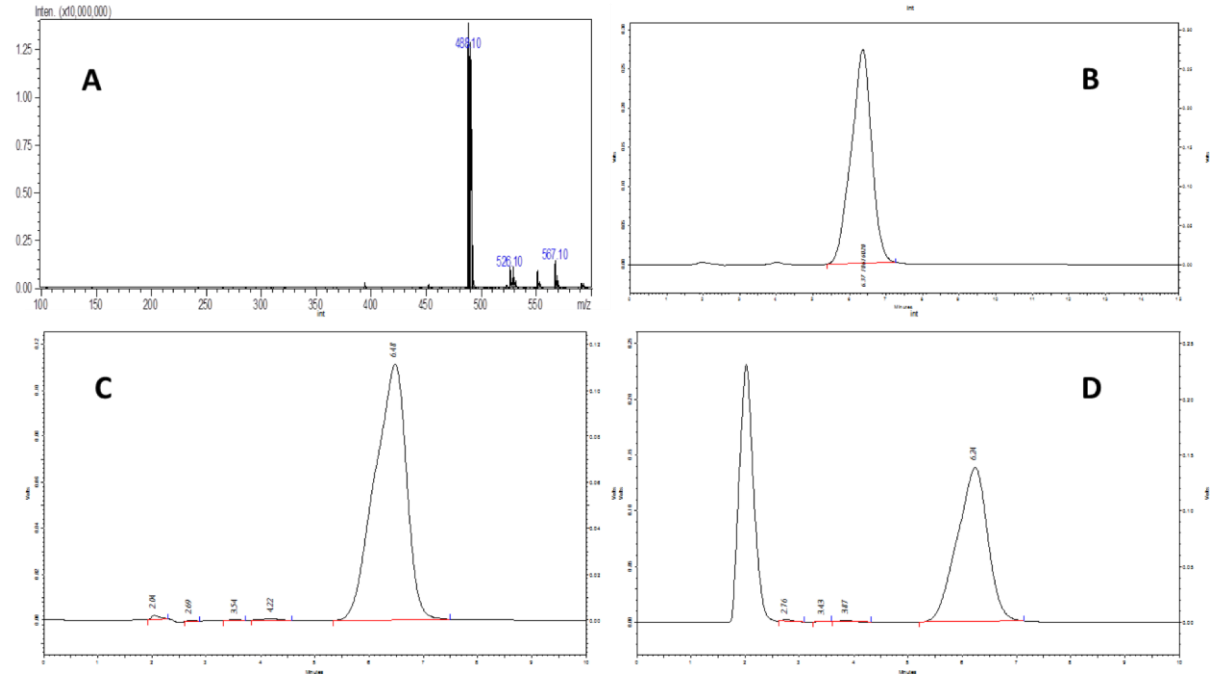

Figure 2: Dasatinib standard mass scan spectra (A), standard LC chromatogram (B), degradation peak of basic hydrolysis (C) and oxidative degradation peak (D)

Chromatographic condition

For the separation Hypersil ODS (150mm x 4.6 mm i.d., 5µ) column using Methanol: 10mM Ammonium Formate as the mobile phase in a isocratic elution at a flow rate of 1.0 mL/min. The column effluents were monitored by a UV detector set at 325 nm. A volume of 20 µL was injected into the system at a run time of 10 min. The operation condition for MS/MS was The Mass parameters were as follows: Probe temperature was ambient, DL temperature was 250°C, Block temperature was set

detection and quantitation, respectively.

RESULTS AND DISCUSSION

Validation

The method was validated over the concentration range of 10–200 µg/mL (r2 = 0.9992) of the drug. The data analysis showed that the % RSD for each concentration was <0.25%. The % RSD for intra- and inter-day precision at three different concentrations, viz., 50, 100, and 200 µg/mL was found to be within the limits (<0.40%) as

Table 2: Degradant observed for Dasatinib from various stress conditions

S.No	Time (hrs)	Basic hydrolysis (% degradation) 0.1N NaOH	Acid hydrolysis (% degradation) 0.1N HCl	Neutral degradation (% degradation) H_20	Oxidative degradation (% degradation) 3% H_20_2	Photo degradation (% degradation) UV
1	0	0	0	0	0	0
2	2	1.73	1.58	1.38	3.87	1.14
3	4	5.82	3.26	3.15	18.74	2.36
4	6	13.29	4.82	5.62	35.53	3.74
5	8	22.67	6.86	7.44	60.26	5.21
6	12	29.14	8.49	8.07	75.14	6.10
7	24	33.36	10.52	10.58	94.52	8.63

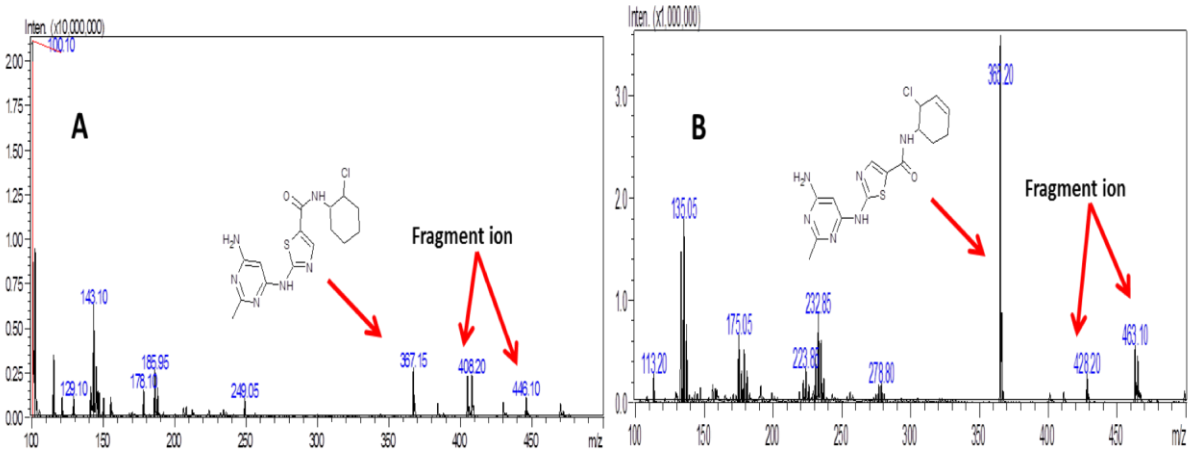

Figure 3: Mass spectra of the degradant NB-I (A) and NB-II (B)

shown in Table 1. Further, stressed samples with spiked known concentration of the drug, produced good recoveries with mean recovery of 100.50% (Table 1). The limits of detection (LOD) and quantification (LOQ) were found to be 10 and 30 ng/mL, respectively. The capacity (k1) and resolution factors (Rs) were found to be within the limits.

Degradation

The degradation behavior of Dasatinib was studied under various stress conditions such as Acidic, Basic, Neutral, Oxidative and photo-degradation as shown in Figure 2. The percentage of the degradant observed from various stress conditions are shown in Table 2.

Acid, Base and Neutral Hydrolysis

Hydrolysis was carried out by diluting a known amount of stock solution with 1N HCl (acid), 1N NaOH (base) and H_2O (neutral) to produce a concentration of 50 µg/mL. Finally the samples were analyzed at different time intervals upto 24 hr and the degradants were recorded and collected.

Oxidative degradation

Oxidative degradation was performed by diluting the

stock solution with 30% H_2O_2 to produce a concentration of 50 µg/mL, which was analyzed at different time intervals.

Photolytic degradation

For photolytic degradation studies stock solution was diluted with water to produce a concentration similar to other studies that is 50 µg/mL. Further the drug substance in solid state was exposed to a temperature of 80°C for 24 hr, which was diluted to produce a concentration of 50 µg/mL and analyzed.

Mass spectral fragmentation

Among the degradations peak produced the degradation peak NB-I and NB-II were the major degradation peak observed in oxidative degradation and basic hydrolysis (Figure 3). For the identification of the molecular ion the separation was done by column (HPLC) and the samples were directly injected into a MS/MS system which consists of a the mobile phase water and acetonitrile in the ratio 20: 80, v/v with the flow rate of 0.5 mL/min, using nitrogen as the drying gas, nebulizer gas, 60 psi; dry temperature, 350°C; capillary voltage, 5kV; vaporizer temperature, 400°C; and dwell time, 200 ms.

CONCLUSION

A stability indicating method has been developed to explore the degradation behavior of Dasatinib by an LC-MS/MS. The method developed was able to separate the degradants from Dasatinib The degradation products formed upon basic hydrolysis (NB-I) may be 2-[(6-amino-2-methylpyrimidin-4-yl)amino]-N-(2-chlorocyclohexyl)-1,3-thiazole-5-carboxamide and degradant

formed during oxidative hydrolysis might be 2-[(6-amino-2-methylpyrimidin-4-yl)amino]-N-(2-chlorocyclohex-3-en-1-yl)-1,3-thiazole-5-carboxamide. Hence the developed method can be used for quality control test and identification of degradation product.

REFERENCES

Antonio D'Avolio, Marco Simiele, Silvia De Francia, Alessandra Ariaudo, Lorena Baietto, Jessica Cusato, Carmen Fava, Giuseppe Saglio, Francesco Di Carlo, Giovanni Di Perri. HPLC MS method for the simultaneous quantification of the antileukemia drugs imatinib, dasatinib and nilotinib in human peripheral blood mononuclear cell (PBMC). J. Pharm. Biomed. Anal.2012; 59: 109-16.

Eva Kralj, JurijTrontelj, TadejPajic, AlbinKristl. Simultaneous measurement of imatinib, nilotinib and dasatinib in dried blood spot by ultrahigh performance liquid chromatography tandem mass spectrometry.J. chromatogr. B. 2012; 903: 150-56.

Haouala. A, Zanolari. B, Rochat.B, Montemurro. M, Zaman.K, Duchosal. M.A, Ris.H.B, Leyvraz. S, Widmer. N, Decosterd. L.A. Therapeutic Drug Monitoring of the new targeted anticancer agentsimatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib by LC tandem mass spectrometry. J. chromatogr. B. 2009; 877:1982-96.

Hassan Karimi-Maleh, AbdollahFallahShojaei, FatemehKarimi, Khalil Tabatabaeian, ShahryarShakeri, Reza Moradi. Simultaneous determination of 6-mercaptopruine, 6-thioguanine and dasatinib as three important anticancer drugs using nanostructure voltammetric sensor employing Pt/MWCNTs and 1-butyl-3-methylimidazoliumhexafluoro phosphate. Biosensors and Bioelectronic.2016; 86: 879-84.

ICH-Q1A (R2). Stability testing of new drug substances and products: Proceeding of international conference on harmonization. February 6, 2003.

ICH-Q2 (R1). Validation of analytical procedures: text and methodology. International Conference on Harmonization, draft revised guidance on Q2 (R1). Fed Regist.March 1, 1995; 60.

Irina Andriamanana, Ines Gana, BénédicteDuretz, Anne Hulin. Simultaneous analysis of anticancer agentsbortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS. J. chromatogr. B. 2013; 926: 83-91.

MarekDziadosz, RüdigerLessig, Heidemarie Bartels. HPLC–DAD protein kinase inhibitor analysis in human serum.J. chromatogr. B. 2012; 893-894: 77-81.

Mhaske. D. V, Dhaneshwar. S. R. Stability Indicating HPTLC and LC Determination of Dasatinib in Pharmaceutical Dosage Form. Chromatographia.2007; 66: 95-102.

Michael T. Furlong, ShrutiAgrawal, Dara Hawthorne, Michael Lago, Steve Unger, Linda Krueger, Bruce Stouffer. A validated LC–MS/MS assay for the simultaneous determination of the anti-leukemic agent dasatinib and two pharmacologically active metabolites in human plasma: Application to a clinical pharmacokinetic study. J. Pharm. and Biomed. Anal.2012; 58: 130-35.

Mohammed G. Kassem, EssamEzzeldin, Hesham M. Korashy, Gamal A.E. Mostafa. High-performance liquid chromatographic method for thedetermination of dasatinib in rabbit plasma using fluorescencedetection and its application to a pharmacokinetic study.J. chromatogr. B. 2013; 939: 73-79.

Silvia De Francia, Antonio D'Avolio, Francesca De Martino, Elisa Pirro, Lorena Baietto, Marco Siccardi, Marco Simiele, Silvia Racca, Giuseppe Saglio, Francesco Di Carlo, Giovanni Di Perri. New HPLC–MS method for the simultaneous quantification of the antileukemia drugs imatinib, dasatinib, and nilotinib in human plasma. J. chromatogr. B. 2009; 877: 1721-26

StéphaneBouchet , Emmanuelle Chauzit, Dominique Ducint, NadègeCastaing, Mireille Canal-Raffin, Nicholas Moore, KarineTitier, Mathieu Molimard. Simultaneous determination of nine tyrosine kinase inhibitors by 96-well solid-phase extraction and ultra-performance LC/MS-MS. Clin.ChimicaActa. 2011; 412: 1060-67.