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Analytical Method development and validation for the Estimation of Dasatinib by Using RP—HPLC Method

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ABSTRACT

A simple and precise method was developed for estimating Dasatinib. The method was found to be specific and precise. The separation was attained on Acquity CSH C18 Column (150*2.0mm & 1.5µm) and linearity was achieved in the concentration rage of 50µg/ml to 250µg/ml of Dasatinib with correlation coefficient 0.99. The percent recovery from the assay was found to be 99.79% for Dasatinib. Limit of detection and quantitation for Dasatinib were within the acceptable range. From the stability studies, the percentage variation was less than 10.0% which is the desired criteria. Therefore, this method can be adopted to estimate Dasatinib in other pharmaceutical formulations.

Keywords: Dasatinib, HPLC, Method development, Linearity, Validation

INRODUCTION

Dasatinib is an oral dual BCR/ABL and Src family tyrosine kinase inhibitor approved for use in patients with chronic myelogenous leukemia (CML).[1] The main targets of Dasatinib, are BCRABL, SRC, Ephrins and GFR. Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Based on modeling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. In vitro, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease.[2, 3] Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression.[4-6] From the literature survey, it was revealed that few UV spectrophotometric method was developed but were not economical. Moreover, RP-HPLC[7-10] and LC-MS[11] and derivative methods were also developed which estimates Dasatinib. In the present research work, a new method was developed to estimate Dasatinib and validated as per ICH guidelines.[12]

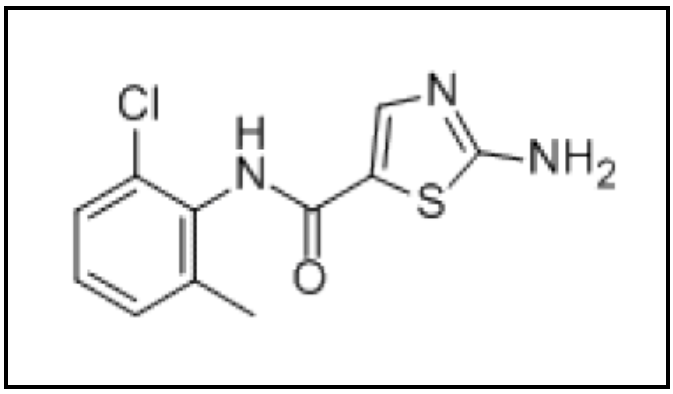

Fig 1: Structure of Dasatinib

MATERIALS AND METHODS

Gift samples of Dasatinib were received from Chandra lab, Hyderabad. KH2PO4 was purchased from Final chemicals whereas water, methanol for HPLC and ortho phosphoric acid were purchased from Merck.

Instrumentation

Waters HPLC was used for the estimation of Dasatinib. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Method Development

Preparation of Standard solution

10 mg of Dasatinib was weighed and exchanged in to 100 ml volumetric jar and broken up in portable stage and after that make up to the check with portable stage and plan 10 µg /ml of arrangement by weakening 1ml to 10ml with portable stage.

Preparation of Sample solution

Sample name: Dasatinib Tablets (Sprycel-100mg). Weigh amount of powder proportionate to 100mg of Dasatinib and exchanged in to 100 ml volumetric carafe and broken up in versatile stage and after that make up to the check with portable stage and plan 10 µg /ml of arrangement by weakening 1ml to 10ml with versatile stage.

Procedure

Mixture of Tri ethylene amine, ACN and Methanol in the ratio 30:50:20 % v/v was used as mobile phase which was injected into the system for 30 minutes prior to injecting the prepared solutions of standard as well as sample. Detection of the drug was achieved at the wavelength of 230nm at room temperature. After several trials, method was optimized followed by validation of the method considering various validation parameters.

RESULTS AND DISCUSSION

Method development was achieved using Acquity CSH C18 Column (150*2.0mm & 1.5µm). Mobile phase was mixture of Tri ethylene amine, ACN and methanol (30:50:20% v/v). Flow rate (1ml/min) and injection volume (10µl) was set. The peaks obtained had good resolution with the retention time 2.090. Chromatogram of optimized trial is shown in figure 2.

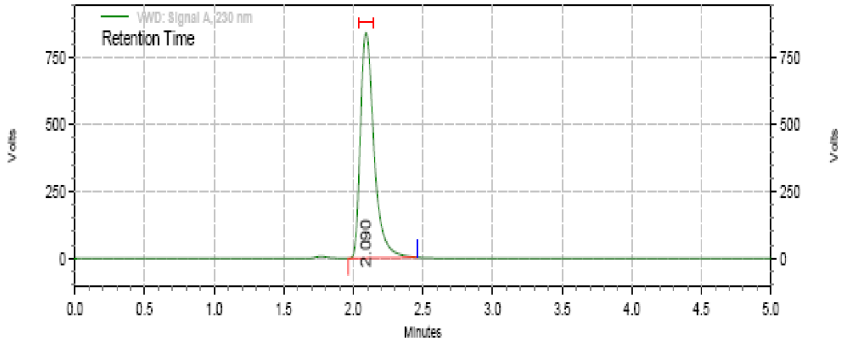

Fig 2: Chromatogram of optimized trial

System Suitability and System Precision

All the parameters were evaluated by performing system suitability studies. The recorded responses for System Suitability & System Precision are depicted in table 1.

Table 1: Results of system suitability parameters

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.091	96343680	2433	1.12
2	2.093	96364770	2415	1.10
3	2.088	96438444	2424	1.11
4	2.086	95994191	2419	1.09
5	2.090	96704491	2463	1.11
6	2.089	96202740	2489	1.13
Mean	2.090	96341386.0	-	-
SD	0.002	237311.884	-	-
%RSD	**0.116**	**0.246**	**-**	**-**

Method validation

Validation of the method was evaluated for various parameters which include linearity, specificity, robustness and stability. The method was also evaluated for specificity of the method and was found to be specific as there were no interactions found. Linearity obtained was shown to have good correlation as shown in table 2.

Table 2: Linearity Relationship

S. No	Parameter	Dasatinib
1	Correlation coefficient	0.9997
2	Slope	32947
3	Intercept	923452

Linearity

The linearity range was observed from 50µg/ml to 250µg/ml of Dasatinib. The respective absorbance values are depicted in table 3. The linearity graph plotted is presented in figure 3.

Table 3: Linearity Results

S. No	Concentration (µg/mL)	Area
1	50	1885.11
2	100	3005.34
3	150	3752.19
4	200	4561.04
5	250	5613.84

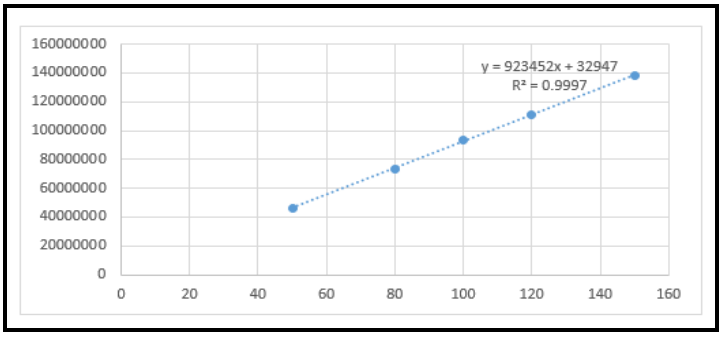

Fig 4: Linearity graph for Dasatinib

Accuracy

Percent recovery of sample solutions at different concentrations (50%, 100%, and 150%) was calculated. The Percent recovery of Dasatinib is depicted in table 4.

Table 4: Accuracy (recovery) data for Dasatinib

Recovery	Area	Avg Area	% Recovery	% Recovered
	46743357			
	46590321			
50	46618082	46650586.67	50.42	100.84
	93917031			
	93857319			
100	93743963	93839437.67	100.64	100.64
	138525998			
	138257065			
150	139250427	138677830.00	150.44	100.29

Limit of Detection and Quantitation

Lowest concentrations of the sample were prepared and measured for LOD and LOQ. The LOD for this method was found to be8.74µg/ml Dasatinib. The LOQ for this method was found to be 26.50µg/ml Dasatinib.

$$LOD = \frac{3.3\sigma}{S}$$

= 3.3 * (87311.88) / 32947

= 8.74µg/ml

$$LOQ = \frac{10\sigma}{S}$$

= 10* (87311.88) / 32947

= 26.50µg/ml

Where

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Robustness

The standard and samples were injected by changing the conditions of chromatography. There was no change observed in the parameters like tailing factor, resolution, plate count and asymmetric factor. Chromatograms for variation in flow rate are presented in figure 5 and 6 whereas chromatograms for variation in temperature are presented in figure 7 and 8. Their respective results are depicted in table 5.

Variation in flow

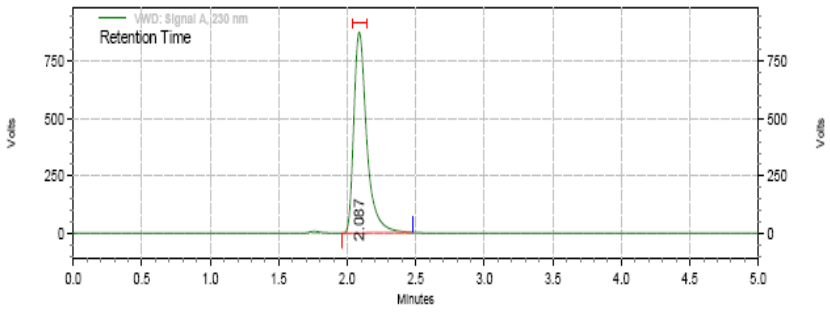

Fig 5: Chromatogram showing less flow

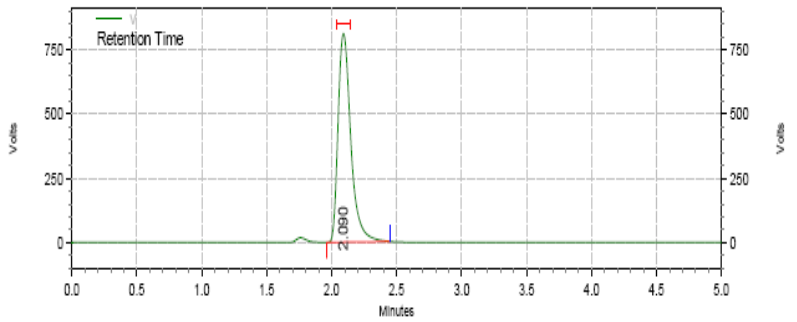

Fig 6: Chromatogram showing more flow

Variation of temperature

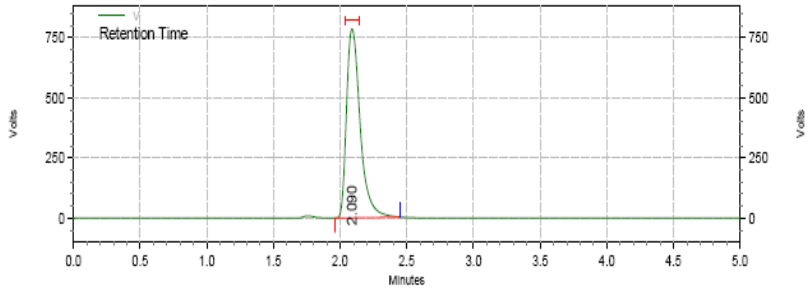

Fig 7: Chromatogram with less temperature

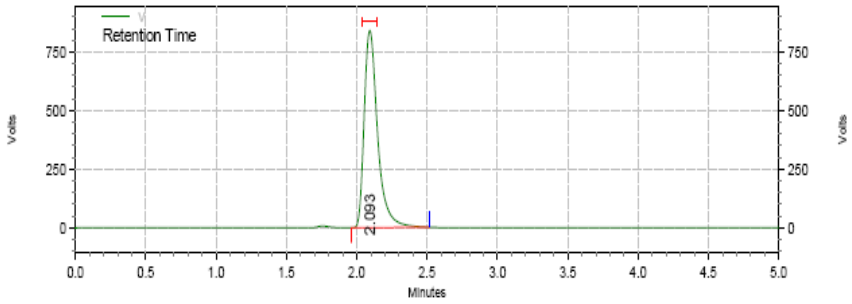

Fig 8: Chromatogram with more temperature

Table 9: Results for variation for Dasatinib

Chromatographic changes		Retention time(min)	Tailing Factor	Theoretical Plates
Flow rate (mL/min)	0.8	2.087	1.74	2456
	1.2	2.090	1.28	2469
Temperature (°C)	35	2.090	1.53	2585
	45	2.093	1.59	2481

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Dasatinib was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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REFERENCE

1. Das J, Chen P, Norris D, Padmanabha R, Lin J, Moquin RV, Shen Z, Cook LS, Doweyko AM, Pitt S, Pang S, Shen DR, Fang Q, de Fex HF, McIntyre KW, Shuster DJ, Gillooly KM, Behnia K, Schieven GL, Wityak J, Barrish JC: 2-aminothiazole as a novel kinase inhibitor template. Structure-activity relationship studies toward the discovery of N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1- piperazinyl)]-2-methyl-4-pyrimidinyl] amino)]-1,3-thiazole-5-carboxamide (dasatinib, BMS-354825) as a potent pan-Src kinase inhibitor. J Med Chem. 2006 Nov 16;49(23):6819-32.
2. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O'Brien S, Nicaise C, Bleickardt E, Blackwood-Chirchir MA, Iyer V, Chen TT, Huang F, Decillis AP, Sawyers CL: Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006 Jun 15;354(24):2531-41.
3. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 3062316, Dasatinib. Retrieved January 13, 2021
4. Keating GM (January 2017). "Dasatinib: A Review in Chronic Myeloid Leukaemia and Ph+ Acute Lymphoblastic Leukaemia". Drugs. 77 (1): 85–96.
5. Olivieri, A.; Manzione, L. (2007). "Dasatinib: a new step in molecular target therapy". Annals of Oncology. 18 Suppl 6: vi42–vi46
6. Yurttaş NO, Eşkazan AE (2018). "Dasatinib-induced pulmonary arterial hypertension". British Journal of Clinical Pharmacology. 84 (5): 835–845.
7. Sankar PR and Anusha S: Development and validation of RP-HPLC method for the determination of Dasatinib in tablet dosage form. Int J Pharm Sci & Res 2019; 10(10): 4531-37
8. Sankar PR and Anusha S: Analytical Method development and Validation of Dasatinib in its Pharmaceutical dosage form by UPLC with Forced Degradation Studies. International Journal for Pharmaceutical Research Scholars. 2013; 2(4): 221-227
9. Alagar Raja. M, Swapna. M, Shirisha. V, David Banji, Rao. K N V, Selva Kumar. D: RP-HPLC method for estimation of dasatinib in active pharmaceutical ingredient and pharmaceutical dosage form as per ich guidelines. Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry. 3(3), 2015, 109- 116
10. Sunil VL, Shahnawaz SS, Shekhawat DS, et al. Rapid and Sensitive RP-HPLC Method for Determination of Potential Genotoxic Impurity in Dasatinib Drug Substance. Int J Chem Sci. 2017;15(4):177
11. Saraner N, Karagoz A, Guney B, Saglam O (2019) Determination of Dasatinib in Human Plasma by Using Liquid Chromatography-Tandem Mass Spectrometry. Int J Analyt Bioanalyt Methods 1:002.
12. ICH Q2B guideline (1996), Validation of Analytical Procedures: Methodology, Guidance for Industry, 1-10.