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Validated RP-HPLC Method for Estimation of Related Impurities in Dasatinib

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Abstract: The analysis of HPLC-UV detector method for the quantification of Dasatinib and its related impurities was proposed in the present investigation. Analysis has been carried by means of reverse phase -HPLC using Waters XTerra MS C-18, (250 × 4.6 mm, 5 µ), and the mobile phase consists of two Channels A and B. Channel-A phosphate Buffer and Channel-B: Methanol and Acetonitrile (10:90). The column temperature was maintained at 40˚C and sample temperature was maintained at ambient and wavelength fixed at 265nm. The flow rate is 1.0 $mL.min^{-1}$. It is found that the proposed method of RP-HPLC with UV-detection for the analysis of Dasatinib impurities are straight forward and applied in qualitative and quantitative analysis. The developed RP-HPLC method was validated as per ICH guidelines with respect to accuracy, linearity, reproducibility, ruggedness, robustness.

Keywords: Dasatinib, Related Substances, Liquid Chromatography, Linearity and Reproducibility.

INTRODUCTION

The chemical name for Dasatinib is N-(2-chloro-6 -methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5thiazolecarboxamide, monohydrate. The molecular formula is $C_{22}H_{26}ClN_7O_2S.H_2O$. Dasatinib [1] is a kinase inhibitor at nanomolar concentrations, inhibits the following kinases BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFRβ. Dasatinib is a white to off-white powder. The drug substance is insoluble in water and slightly soluble in ethanol and methanol. The chemical structure of Dasatinib was shown in **(Fig.1).**

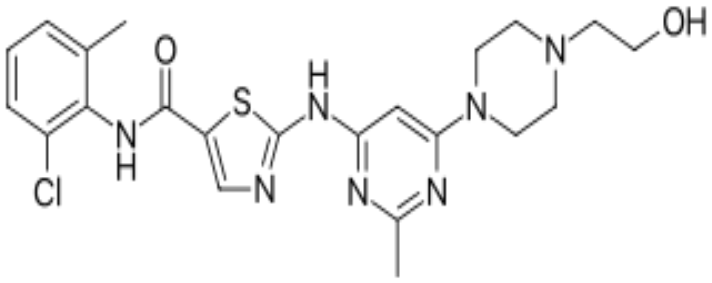

Fig.1: Chemical structure of Dasatinib

Impurity profiling of active pharmaceutical ingredients (API) in both bulk material and formulations is one of the most challenging tasks. As per the requirements of various regulatory authorities, the impurity profile study of drug substances and drug products has to be carried out using a suitable analytical method in the final product. [2-3].The chemical structures of impurities (A-E) were shown in **Fig.2-6**.

The literature review reveals that very few of analytical methods reported till to date for estimation of Dasatinib by various techniques like HPLC [4-8], LC-MS/MS [9-10]. Hence, the objective of present study

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is to develop simple, precise and accurate RP-HPLC method for estimation of related substances in Dasatinib in pharmaceutical dosage forms. The developed method was validated according ICH [11-12] guidelines including various stability parameters proved for its accuracy.

Related Substance (Impurities) Structures

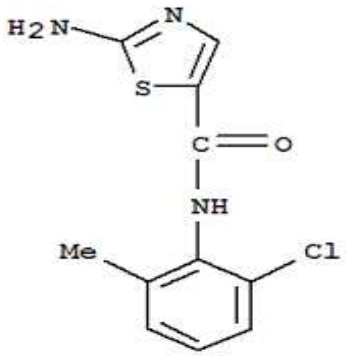

Fig.2: Chemical structure of 2-amino-N-(2-chloro-6methyl phenyl) thiazole-5-carbaxamide (Impurity-A)

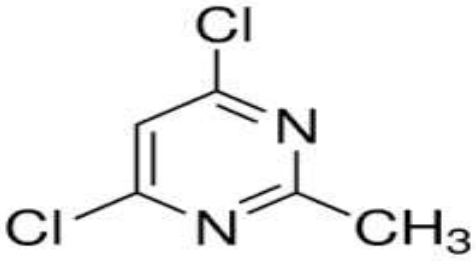

Fig.3: Chemical structure of 4, 6-dicholoro-2-methyl pyridine (Impurity-B)

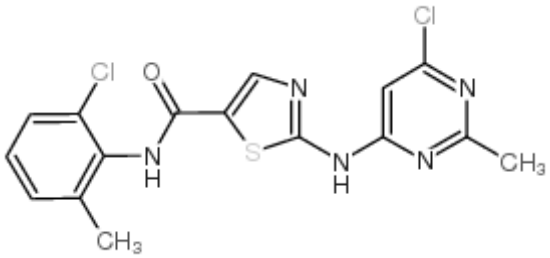

Fig.4: Chemical structure of (2-(6-chloro-2-methylpyrimidin-4-ylamino)-N-(2-chloro-6-methyl phenyl) thiazole-5- carbaxamide) (Impurity-C)

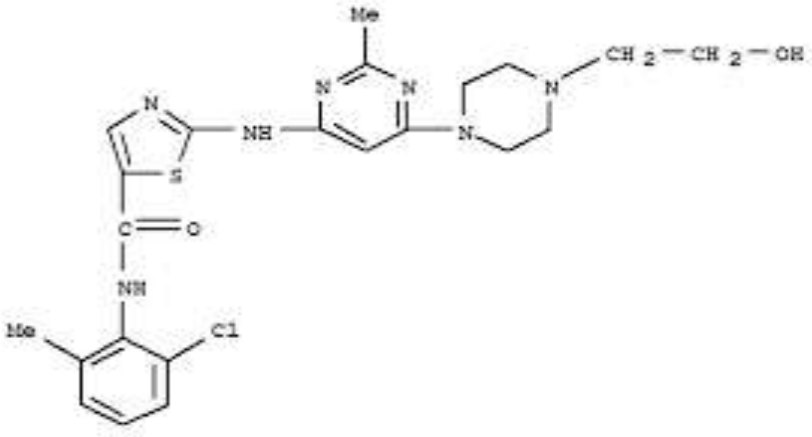

Fig.5: Chemical structure of N-(2-chloro-6-methyl phenyl)-2-[(6-(2-hydroxyethyl)-1-piperazinyl)-2-methyl-4-pyrimidinyl] amino] -5-thiazole carboxamide (Impurity-D)

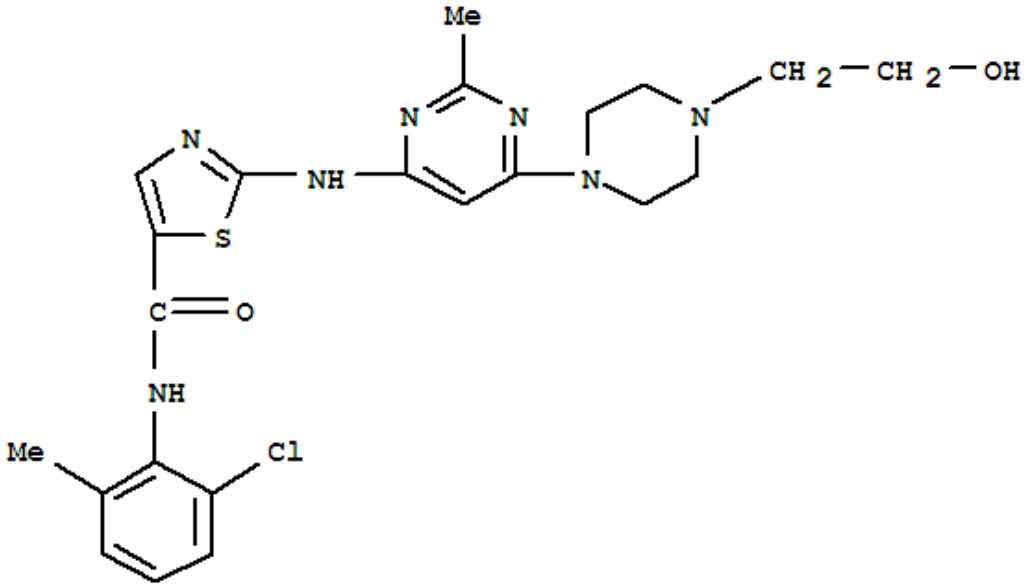

Fig.6: Chemical structure of N-(2-Chloro-6-methylphenyl)-2-({6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl} amino)-1, 3-thiazole-5-carboxamide (Impurity-E)

EXPERIMENTAL

1) Reagents and chemicals

Sodium dihydrogen orthophosphate, Disodium hydrogen orthophosphate, Acetonitrile, Methanol and Dimethyl sulfoxide were procured from Merck, Mumbai, and Water (Milli-Q). All chemicals were of an analytical grade and used as received.

2) Instrumentation

Waters HPLC system with PDA detector was used for analytical separations. The analytical column was waters XTerra MS C-18, (250 × 4.6 mm, 5 µ), the mobile phase consists of two Channels A and B. Channel-A phosphate Buffer and Channel-B: Methanol and Acetonitrile (10:90) were used. The flow rate is 1.0 $mL.min^{-1}$. The column temperature was maintained at 40˚C and sample temperature was maintained at ambient and wavelength fixed at 265nm and injection volume was 10µL. $Empower^3$ software was used for the control of HPLC system and data collection.

3) Preparation of Reference Solution and Sample Solution

Reference Solution Preparation

15 mg of each impurity (Impurity-A, B, C, D and E) was accurately weighed and 10.0 mg of Dasatinib standard transferred into a 100 mL volumetric flask, 5mL of dimethyl sulfoxide was added and sonicated. Then volume was made up with diluent. 1.0mL of stock solution was pipette out into a 100 mL volumetric flask and finally volume was made up with diluent.

Sample Preparation

Weighed accurately 100 mg of Dasatinib sample transferred into a 100 mL volumetric flask, 50mL of diluent was added and sonicated. Then volume was made up with diluent.

Spiked Sample Preparation

Impurity Stock Solution

Weighed accurately 15 mg of each impurity (Impurity-A, B, C, D & E) transferred into a 50 mL volumetric flask, 5mL of dimethyl sulfoxide was added and sonicated.

100 mg of Dasatinib sample weighed accurately and transferred into a 100 mL volumetric flask, 50mL of diluent was added and sonicated to dissolve, further 1.0 mL of impurity stock solution added mixed well. Then volume was made up to 100 mL with diluent.

RESULTS AND DISCUSSION

Optimization Characteristics

For successful RP-HPLC method development, the nature of API (functionality, acidity, or basicity), related impurities, synthetic process, the possible degradation pathways and their degradation products is thoroughly understood. In addition, successful method development should result a robust, simple, precise, accurate and time efficient method.

Selection of Wavelength

The sensitivity of the HPLC method depends upon the selection of detection wavelength. An ideal wavelength is one that gives good response for related substances and the drugs to be detected. The wavelength for measurement was selected as 265 nm from the absorption spectrum.

Selection of Stationary Phase

Selection of the proper stationary phase depends up on the nature of the drug selected. Based on the literature survey, different C_{18} columns could be appropriately used for the separation of related substances for Dasatinib. Hence, Waters XTerra MS C-18 was selected in the proposed method

Selection of Mobile Phase

Various mobile phase and stationary phases were employed to develop a suitable HPLC method for the quantitative determination of impurities in Dasatinib. In the first trail, poor peak shape and resolution was observed when Inertsil ODS-3V (250x4.6mm, 5um) and gradient mobile phase programmed of Mobile Phase: A (10Mm phosphate buffer) and Mobile Phase: B (Acetonitrile and Methanol in the ratio 50.50). There was no proper resolution of impurities and analyte peak and efficiency of the peak is also not achieved and peak interferences are present.

In second attempt made using waters XTerra MS C-18, (250 × 4.6 mm, 5 µ), column and gradient mobile phase programmed of mobile phase: A (10Mm phosphate buffer) and mobile phase: B (Acetonitrile and Methanol in the ratio 10:90) the resolution of both drug and impurities was achieved. These chromatographic conditions were selected for validation studies. The system suitability results obtained using these chromatographic conditions were incorporated in **Table. 1**. The standard chromatogram was shown in **Fig. 7**.

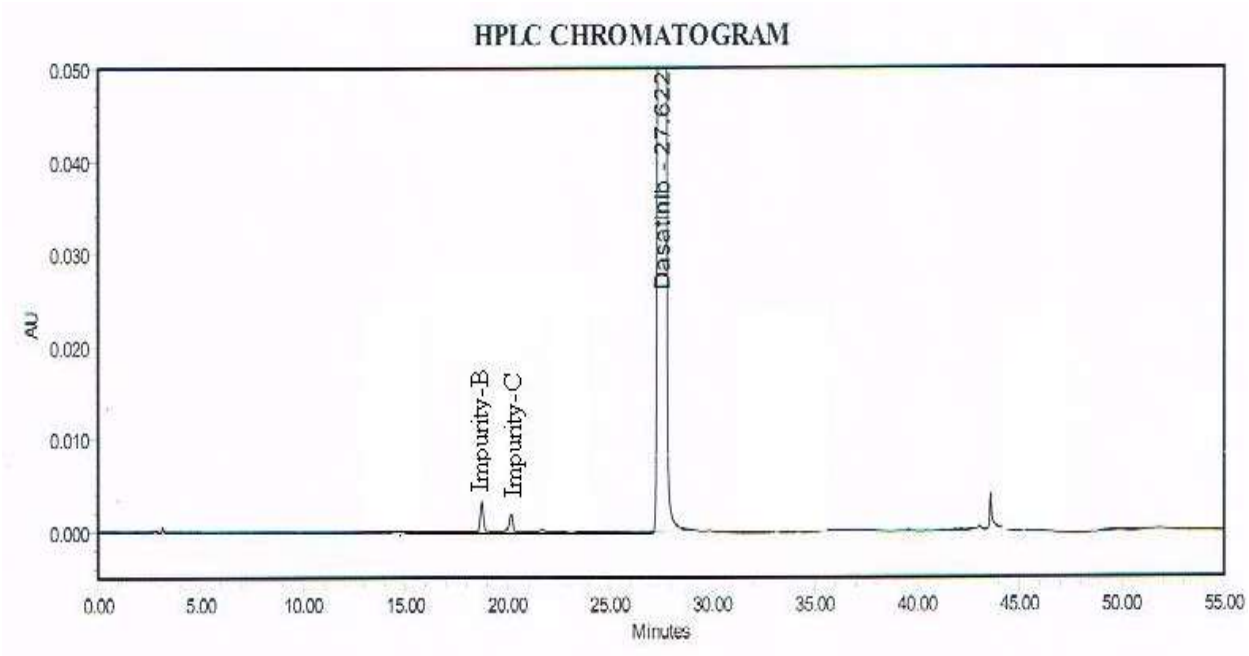

Fig.7: HPLC chromatogram for system suitability

Table 1: Summary for system suitability parameters

Resolution between Impurity-B and Impurity-C	Tailing factor for Dasatinib	Theoretical plates for Dasatinib
5.95	1.45	36512

METHOD VALIDATION

1) Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected as per the test method. Typical chromatogram of blank was shown in **Fig.8.**

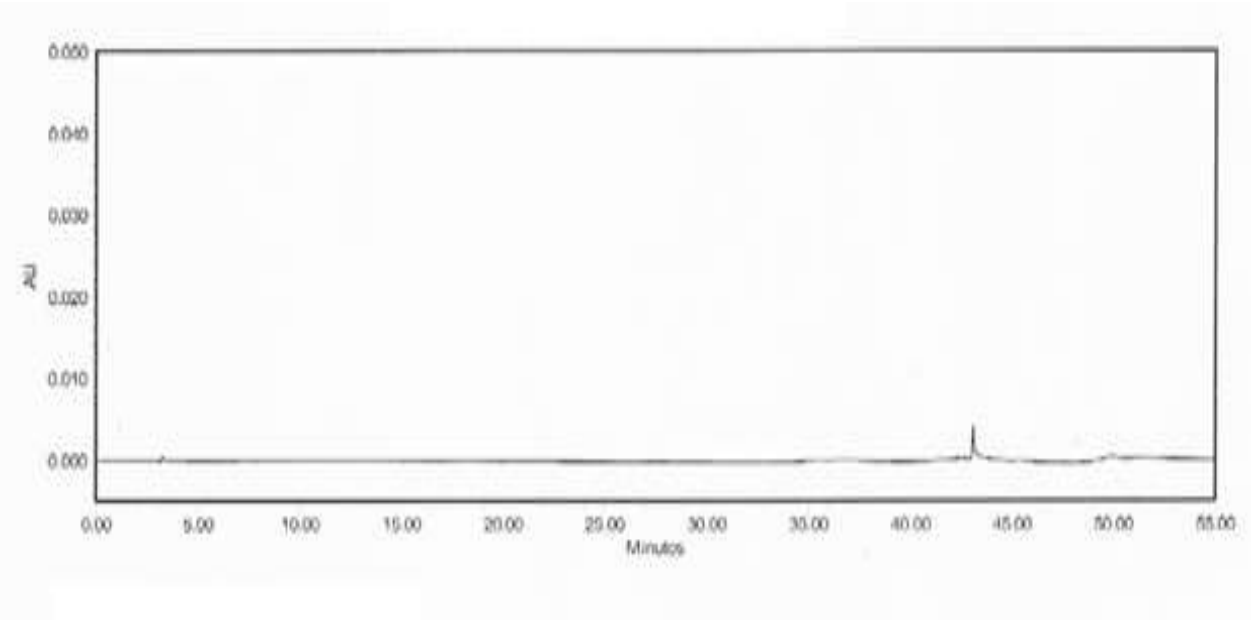

Fig.8: Typical chromatogram of blank

Impurity Interference

A study to establish the impurity interference was established. It was observed that known impurities are not co eluting with each other and main analyte peak. Dasatinib standard solution preparation and in spiked test preparation was calculated and found to be within the acceptable limit.Typical chromatogram of spiked sample was incorporated in **Fig.9.** Impurity interference data was mentioned in the **Table.2.**

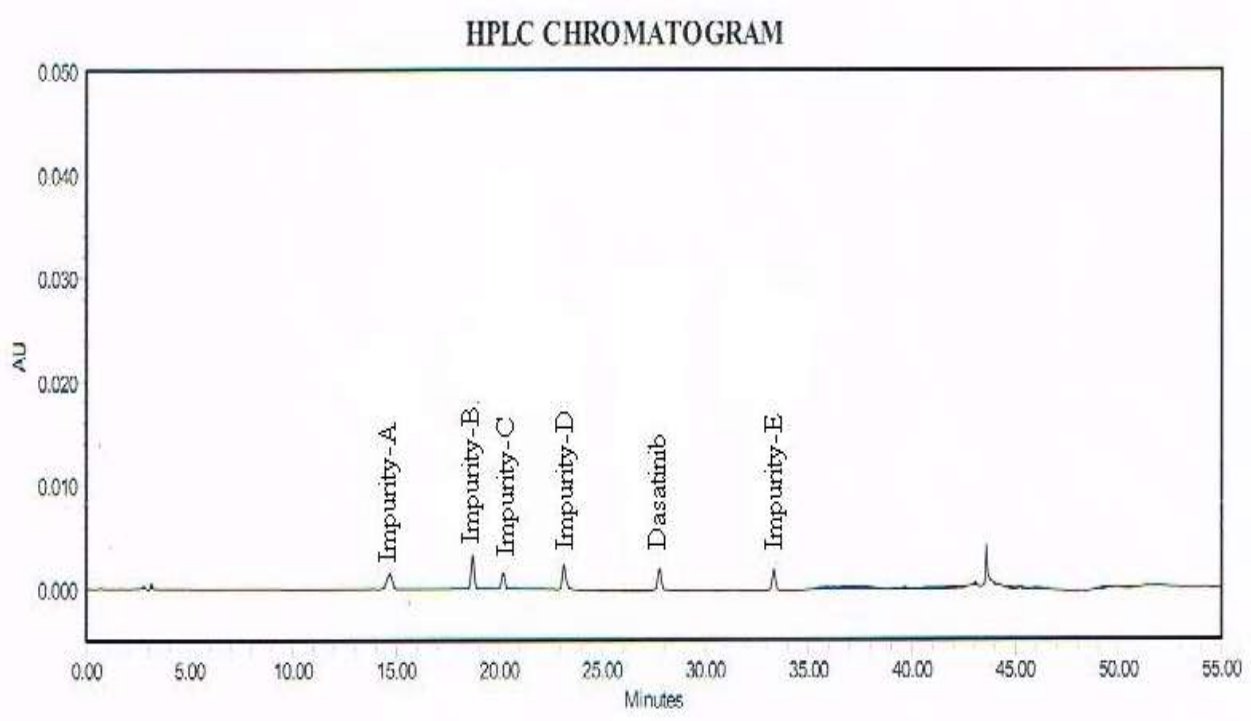

Fig.9: Typical chromatogram spiked sample

Table 2: Impurity interference data

Preparation	RT	Peak response
Blank	NA	NA
Sample with spike impurities		
Impurity-A	14.69	26861
Impurity-B	18.71	33604
Impurity-C	20.21	18837
Impurity-D	23.14	28829
Impurity-E	33.33	21439
Dasatinib	27.78	24844

2) Method Precision

Precision of the impurities was determined by injecting six sample solutions spiked with impurities (A, B, C, D and E) at specification level.

Table 3: Results of Method Precision

Area response					
Sample No	**Impurity-A**	**Impurity-B**	**Impurity-C**	**Impurity-D**	**Impurity-E**
1	26349	32988	18844	28996	21497
2	26350	33065	18769	29237	21535
3	26308	32973	18704	28874	21566
4	26318	32978	18717	29137	21569
5	26283	32896	18713	29013	21608
6	26419	32951	18649	29065	21585
Mean	**26338**	**32975**	**18733**	**29064**	**21565**
% RSD	**0.18**	**0.17**	**0.36**	**0.46**	**0.20**

The method precession was performed with six replicate solutions of standard solutions prepared and the system suitability parameters found were within the acceptance criteria. The samples were prepared as per the method and the results for precision study were presented in **Table.3.**

3) Limit of Detection (LOQ) & Limit of Quantitation (LOD)

The limit of detection and limit of quantitation values obtained for each impurity and Dasatinib are within the acceptance criteria. The details of LOD and LOQ results were incorporated in **Table. 4** and **Table. 5.**

4) Linearity and Range

Standard solutions of Dasatinib, impurities in the concentration levels from LOQ to 200 % of standard solution were injected into HPLC system. The linearity graph was plotted from LOQ to 200% of drug concentration. Results obtained were tabulated in **Table.6 and Table.7**.

Table 4: LOQ for Dasatinib and impurities

Name	Concentration $\mu g.mL^{-1}$	Signal to noise ratio
Dasatinib	0.185	10.56
Impurity-A	0.408	11.35
Impurity-B	0.180	10.98
Impurity-C	0.336	12.15
Impurity-D	0.252	11.65
Impurity-E	0.300	10.22

Table 5: LOD for Dasatinib and impurities

Name	Concentration μg.mL^{-1}	Signal to noise ratio
Dasatinib	0.061	3.49
Impurity-A	0.135	3.43
Impurity-B	0.059	2.98
Impurity-C	0.111	3.96
Impurity-D	0.083	3.55
Impurity-E	0.099	3.10

Table 6: Linearity data of Dasatinib and impurities

Sample No.	% level	Peak area				
		Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E
1	LOQ	3483	1997	1947	2072	2176
2	25	6393	8182	4123	6728	5169
3	50	12707	16226	8842	13628	10171
4	75	19812	24249	13088	21173	14429
5	100	26476	32727	17916	27996	20140
6	125	33605	41353	22664	35510	24120
7	150	39409	49410	27157	42796	28805
8	200	53835	69931	36536	58533	37849

Table 7: Regression analysis data of impurities

Linear regression analysis	Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E
Correlation coefficient (r^2)	0.9998	1.0000	0.9999	0.9998	0.9998
%Y- intercept	-1.63	-0.54	-2.21	-2.94	2.90
Slope	8989166	110112109	6132431	9772953	6266754

5) Accuracy

Recovery of impurities in Dasatinib was performed. The sample was taken and varying amounts of Dasatinib impurities representing 50 to 200 % of specification level were added to the flasks. The spiked samples were prepared as per the method and the results are tabulated in **Table.8.**

Table 8: Accuracy study of Dasatinib

S.No.	Theoretical (%)	% Mean Recovery				
		Impurity-A	Impurity-B	Impurity-C	Impurity-D	Impurity-E
1	50	106.6	94.5	103.2	104.1	104.6
2	100	108.0	95.3	101.7	100.6	90.4
3	150	104.1	97.7	99.8	98.2	90.2
4	200	103.8	100.6	98.9	101.9	89.7

RESULTS & DISCUSSION

An accurate, simple and precise HPLC method was successfully developed. The results obtained were accurate and reproducible. For Selectivity, the chromatograms were recorded for standard and sample solutions of Dasatinib and its related substances. Selectivity studies reveal that the peak is well separated from each other. The linearity results for Dasatinib and all the impurities in the specified concentration range are found satisfactory, with a correlation coefficient greater than 0.99.Calibration curve was plotted and correlation co-efficient for Dasatinib and its impurities found to be 0.9998, 1.0000, 0.9999, 0.9998 and 0.9998 respectively.

The limit of detection (LOD) and limit of quantitation (LOQ) for Dasatinib found to be 0.061-0.185 $\mu g.mL^{-1}$, for impurity-A 0.135-0.408 $\mu g.mL^{-1}$, for impurity-B 0.059-0.180 $\mu g.mL^{-1}$, for impurity-C 0.111-0.336 $\mu g.mL^{-1}$, for impurity-D 0.083-0.252 $\mu g.mL^{-1}$ and for impurity-E 0.099-0.300 $\mu g.mL^{-1}$ respectively. The accuracy studies were shown as % recovery for Dasatinib and its impurities at 50 to 200%.The limit of % recovered shown is in the range of 90 and 110% and the results obtained were found to be within the limits. For Precision studies six replicate injections were performed. %RSD was determined from the peak areas of Dasatinib and its impurities. The acceptance limit should be not more than 10, and the results were found to be within the acceptance limits.

CONCUSIONS

A simple and precise RP-HPLC method has been developed by the author for the assay the related substances of Dasatinib in active pharmaceutical ingredient (API). From the results, it was observed that the developed method was proven to be specific, linear, accurate, rugged and robust and is suitable for its intended purpose. Hence it was concluded that this method could be used for the routine estimation of related substances of Dasatinib in bulk and pharmaceutical dosage form.

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