DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR QUANTITATIVE ESTIMATION OF DASATINIB IN DASATINIB TABLET DOSAGE FORM

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid Chromatography assay method has been developed for estimation of Dasatinib in tablet formulations. The separation was achieved by using column Zorbax SB-C18 (150x4.6mm, 3.5µ). The mobile phase-A consisted of pH 5.8 phosphate buffer and Acetonitrile in the ratio of (90:10 v/v) and mobile phase-B consisted of Water and Acetonitrile in the ratio of (10:90 v/v). The flow rate was 1.2 mL/min, column oven temperature 45°C and sampler cooler was maintained 25°C respectively and the Dasatinib was detected using UV detector at the wavelength of 320 nm and injection volume was 10µL. The retention time of Dasatinib was noted to be 4.10 min respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: Dasatinib, Liquid chromatography, Force Degradation and Validation.

1.0 Introduction

Dasatinib is an inhibitor of multiple tyrosine kinases. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines over expressing BCR-ABL. The chemical name for Dasatinib is N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazole carboxamide. The molecular formula is $C_{22}H_{26}ClN_7O_2S.H_2O$, which corresponds to a formula weight of 506.02 (monohydrate). The anhydrous free base has a molecular weight of 488.01. Dasatinib is a white to off-white powder. The drug substance is soluble in dimethyl sulphoxide and practically insoluble in water and slightly soluble in ethanol and methanol. The molecule structure is shown in **Figure: 1.1**

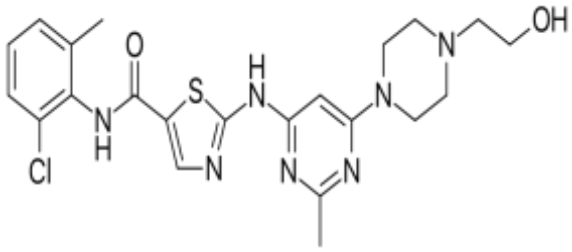

Figure.1.1 Structure of Dasatinib

A few analytical methods have been reported for quantitative estimationof Dasatinib in pharmaceutical formulations. A thorough literature survey of Dasatinib revealed that very few analytical methods had been reported for estimation of Dasatinib hitherto. Majority of methods for determination of Dasatinib in biological fluids and pharmaceutical dosage forms includes LC-MS/MS [1-4], LC-MS [5-6], HPTLC-LC [7], HPTLC [8], UPLC-MS [9], HPLC-MS [10], RP-HPLC [11] and UV-Visible Spectrophotometric method [12-13].

The objective of the present work is to develop a stability indicating HPLC method and validated as per ICH [14-18] and Q2(R1) validation guidelines.

2.0 Experimental

Chemicals and reagents:

Potassium dihydrogen orthophosphate, potassium hydroxide pellets, orthophosphoric acid, Acetonitrile and water was from Merck chemicals Mumbai, India. Mumbai, India. 0.45µm PVDF filter and 0.45µ Nylon membrane filter were from Millex-HN, Millipore Mumbai, India.

Instruments and equipments

Agilent HPLC model:1260 with DAD, open lab software, Waters alliance e2695, Empower^3 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were used.

Preparation of 10% Potassium hydroxide solution:

Weighed 10.0145 g of potassium hydroxide pellets and transferred into a 100 mL volumetric flask, added 50 mL of water, sonicated to dissolve, made up the volume with water and mixed well.

Preparation of pH 5.8 buffer solution:

Weighed 2.73508 g of Potassium dihydrogen phosphate and transferred into a 2000 mL of water and mixed well. Adjusted the pH to 5.82 with 10% potassium hydroxide solution and mixed well. Filtered through 0.45 µm membrane filter and sonicated to degas.

Preparation of pH 3.0 buffer solution:

Weighed 2.75451 g of Potassium dihydrogen phosphate and transferred into a 1000 mL of water and mixed well. Adjusted the pH to 3.03 with diluted orthophosphoric acid and mixed well. Filtered through 0.45 µm membrane filter and sonicated to degas.

Preparation of mobile phase-A:

Prepared a mixture of 900 mL of pH 5.8 buffer solution and 100 mL of Acetonitrile in the ratio of 90:10 (% v/v) and sonicated to degas.

Preparation of mobile phase-B:

Prepared a mixture of 900 mL of acetonitrile and 100 mL of water in the ratio of 90:10 (%v/v) and sonicated to degas.

Preparation of diluent:

Prepared a mixture of 1400 mL of methanol and 600 mL of pH3.0 buffer solution in the ratio of 70:30 (%v/v) and sonicated to degas.

Preparation of standard solution:

Weighed accurately 50.54 mg of Dasatinib working standard in to a 50 mL volumetric flask, added 30 mL diluent, sonicated for 5 minutes to dissolve, made up the volume with diluent and mixed well. Further transferred 5.0 mL of this solution into a 50 mL volumetric flask, diluted to volume with diluent and mixed well. (The concentration of the solution contains 0.1 mg/ mL of Dasatinib).

Preparation of test solution:

Weighed 10tablets, taken average weight and crushed into fine powder. Weighed accurately 211.95 mg of sample powder (equivalent to 50 mg of Dasatinib) and transferred into 100 mL volumetric flask, added 70 mL of diluent, sonicated for 30 minutes with intermediate shaking to dissolve, maintaining the temperature at 25°C, then diluted to the volume with diluent and mixed well. Filtered the solution through 0.45 µm PVDF syringe filter and discarded first 2 mL of filtrate. Transferred 5.0 mL of filtrate test solution into 25 mL volumetric flask, diluted to the volume with diluent and mixed well.(The concentration of the solution contains 0.1 mg/ mL of Dasatinib).

Preparation of placebo solution:

Weighed accurately 162.24 mg of Dasatinib placebo powder (equivalent to 50 mg of Dasatinib) and transferred into 100 mL volumetric flask, added 70 mL of diluent, sonicated for 30 minutes with intermediate shaking to dissolve, maintaining the temperature at 25°C, then diluted to the volume with diluent and mixed well. Filtered the solution through 0.45 µm PVDF syringe filter and discarded first 2 mL of filtrate. Transferred 5.0 mL of filtrate placebo solution into 25 mL

volumetric flask, diluted to the volume with diluent and mixed well.(The concentration of the solution contains 0.1 mg/ mL of Dasatinib).

Chromatographic conditions:

Chromatographic analysis was performed on Zorbax SB-C18 150x4.6mm, 3.5µ column. The mobile phase-A consisted of pH 5.8 phosphate buffer and Acetonitrile in the ratio of (90:10 v/v) and mobile phase-B consisted of Water and Acetonitrile in the ratio of (10:90 v/v). The flow rate was 1.2mL/min, column oven temperature 45°C and sample cooler 25°C, the injection volume was 10µL, and detection was performed at 320 nm using a photodiode array detector (PDA).

3.0 Method development:

Spectroscopic analysis of compound Dasatinib showed that maximum UV absorbance (λmax) at 320 nm respectively. To develop a suitable and robust LC method for the determination of Dasatinib, different mobile phases were employed to achieve the best separation and resolution.

Different mobile phase and stationary phases were employed to develop a suitable LC method for the quantitative determination of Dasatinib. A number of column chemistries supplied by different manufacturers and different mobile phase composition were tried to get good peak shape and selectivity for the Dasatinib.

Poor peak shape and resolution was observed when Inertsil ODS-3V (150mm x 4.6mm, 5µ) and gradient mobile phase programmed of mobile phase-A pH 5.80 phosphate buffer and acetonitrile. Mobile phase-B acetonitrile and water. There was no proper peak shape was observed.

In second attempt made using Zorbax SB-C18, 150 x 4.6 mm, 3.5µm column, and gradient mobile phase programmed of mobile Phase: A pH 5.80 phosphate buffer: acetonitrile and mobile phase-B acetonitrile: water.

After conducting above trials it was found that isocratic mode is not at all useful in this case so moved to gradient mode of separation to get good separation and also particle size of the column decreased to 3.5µ from 5µ to get good separation. To prevent broadening of peak shape injection volume was decreased to 10µL from 20 µL because higher injection volume leads to fronting.

Peak shape of Dasatinib was found good and all impurity peaks were well separated from the main peak. Hence this method was finalized to conduct further studies and validation parameters according to ICH guidelines. The chromatogram of Dasatinib standard using the proposed method is shown in **(Figure: 1.2)** system suitability results of the method are presented in **Table-1.2.**

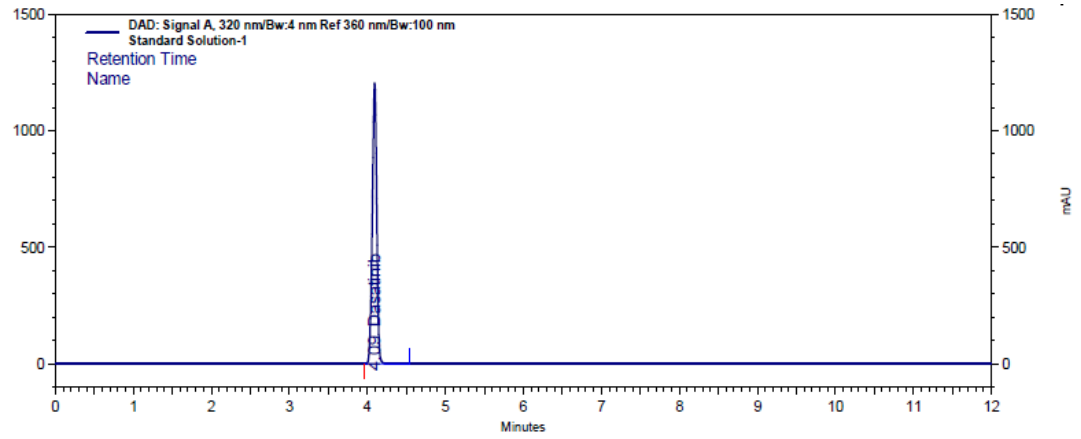

Figure 1.2: Typical chromatogram of standard

4.0 Method validation:

The developed RP-HPLC method extensively validated for assay of Dasatinib using the following parameters.

4.1 Specificity:

Blank and placebo interference:

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. **Figure: 1.3 & 1.4.**

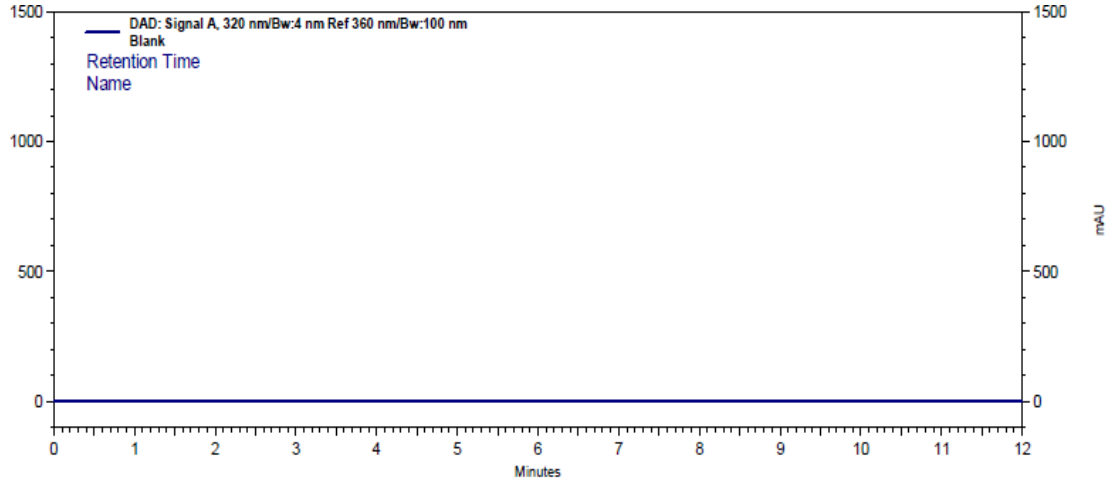

Figure: 1.3 Typical chromatogram of blank

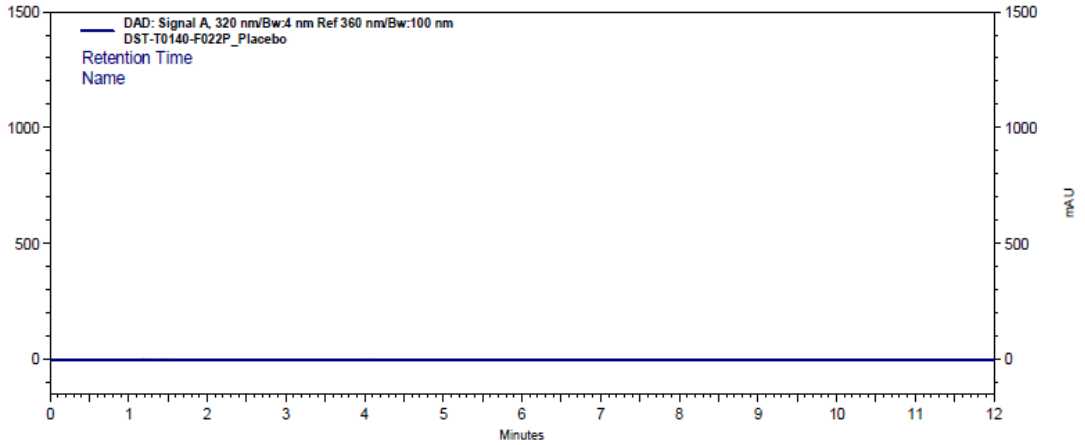

Figure: 1.4 Typical chromatogram of placebo

Table. 1.1 Specificity results for Dasatinib

S.No	Name	Retention Time (min)
1	Blank	ND
2	Placebo solution	ND
3	Standard solution	4.09
4	Sample solution	4.10

The chromatogram of blank and placebo are not showing any peak at the retention time of Dasatinib.

Table. 1.2 System suitability parameters for Dasatinib

Name of the Component	Retention Time	Theoretical plates	Tailing factor
Dasatinib	4.09	23670	1.0

Force Degradation studies:

Table: 1.3 Forced degradation results of Dasatinib

Stress Type	Degradation condition	%Assay	% Degradation
As such	Controlled sample	102.5	NA
Oxidative degradation	30% H_2O_2solution and heat it at 80ºC for 3 hours	97.1	5.44
Acid degradation	5.0 N HCl and heat it at 80ºC for 3 hours	102.4	0.09
Alkali degradation	5.0 N NaOH and heat it at 80ºC for 3 hours	102.4	0.14
Thermal degradation	60ºC in oven for 7 days	102.1	0.4
Humidity degradation	40ºC and 75% RH for 7 days	102.2	0.33
Photolytic degradation	Photolytic degradation Exposure to 1.2 million lux hours at 200 watt hours/square meter ultra violet energy	102.4	0.11

Significant degradation was observed in oxidative stress condition. Hence it can be concluded that Dasatinib tablets is sensitive to oxidation.

4.2 Precision

4.2.1 System Precision:

Perform the study of standard five times and determine the %RSD of peak area of replicate injections of Dasatinib.

Table: 1.4 System Precision data for Dasatinib

Injection No	Dasatinib
1	10058924
2	10068935
3	10053709
4	10069228
5	10065585
Mean area	**10063276**
SD	**6768.4832**
%RSD	**0.1**

The %RSD of peak area for Dasatinib was found to be 0.1% which is below 2.0% indicates that the system gives precise result.

4.2.2 Method precision:

The precision of test method was evaluated by doing assay for six samples of Dasatinib tablets as per test method. The content in mg and % label claim for Dasatinib for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The chromatogram was shown in **Figure: 1.5** and data were shown in **Table: 1.5.**

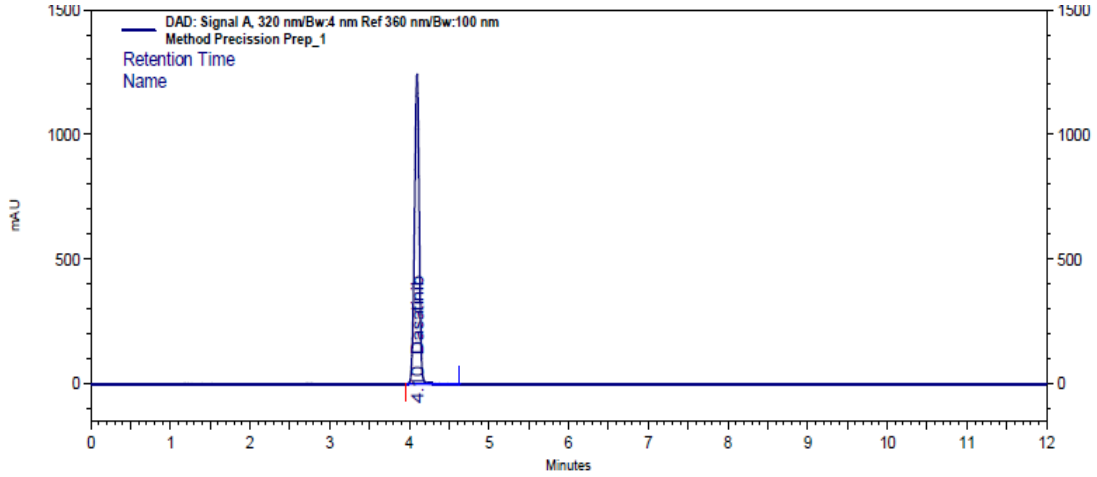

Fig: 1.5 Method precision sample chromatogram

Table: 1.5 Method precision data for Dasatinib

No. of preparations	% Assay
1	103.5
2	103.6
3	103.7
4	101.9
5	101.2
6	100.8
Average	**102.5**
STDEV	**1.3096**
%RSD	**1.3**

4.3 Linearity:

The standard curve was obtained in the concentration range of 50.0789-150.2368 µg/mL for Dasatinib. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in **Figure: 1.6** to demonstrate the linearity of the proposed method. From the data obtained which given in **Table: 1.6** the method was found to be linear within the proposed range.

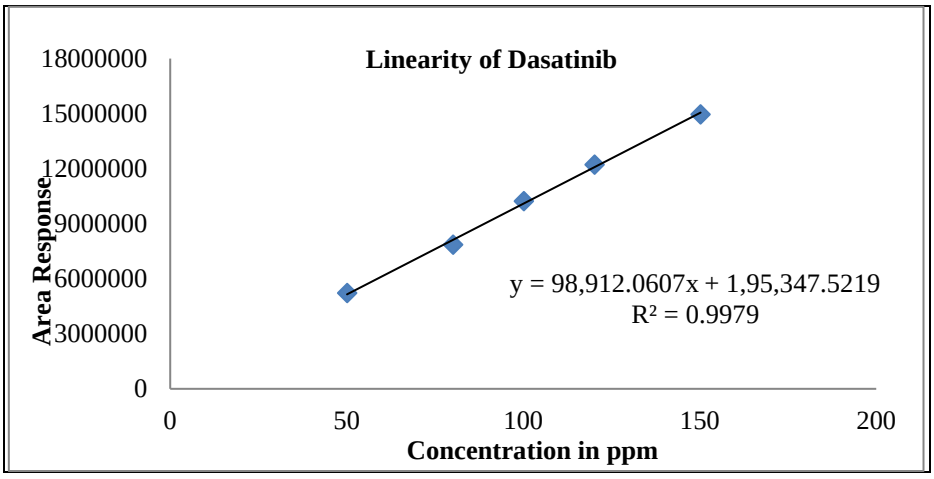

Figure: 1.6 Calibration curve for Dasatinib

Table: 1.6 Linearity studies for Dasatinib by proposed method

S.No	Linearity Level (%)	Concentration (µg/mL)	Area
1	50	50.0789	5224094
2	75	80.1263	7854779
3	100	100.1479	10242511
4	120	120.1895	12217541
5	150	150.2368	14970935
Slope			98912.061
Intercept			195347.522
R^2			0.9979
% Y-Intercept			1.91

4.4 Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Dasatinib, analyzed as per the proposed method. The percentage recoveries with found in the range of 98.8 to 101.1 for Dasatinib. The data obtained which given in **Table:1.7** the method was found to be accurate.

Table: 1.7 Recovery studies for Dasatinib by proposed method

Recovery Level	Amount Added (mg)	Amount recovered (mg)	% Recovery	% Mean Accuracy
50%	50.2744	50.9697	101.4	101.1
	50.2941	50.8961	101.2	
	50.629	50.9659	100.7	
100%	100.5094	101.7399	101.2	101
	100.8049	101.6055	100.8	
	100.7064	101.6855	101	
150%	150.0943	148.7554	99.1	98.8
	149.9367	148.572	99.1	
	150.705	148.1129	98.3	

4.5 Solution stability of analytical solutions:

Standard and sample solutions were kept for about 48 hrs at room temperature in transparent bottles in auto sampler and in refrigerator 2-8°C. The stability of standard and sample solutions was determined by comparison of "old" prepared standard solutions with freshly prepared standard solutions.

Table:1.8 Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
24hrs	1.08	1.02
48hrs	1.06	1.04

Table:1.9 Results for solution stability of sample at room temperature

Time Interval	%Assay	%Assay difference
Initial	102.5	NA
24hrs	103.8	1.3
48hrs	105.5	3.0

Table: 1.10 Results for solution stability of sample in Refrigerator

Time Interval	**%Assay**	**% Assay difference**
Initial	102.5	NA
24hrs	103.0	0.5
48hrs	103.6	1.1

4.6 Filter validation:

Performed the filter validation for sample solution, one portion of the solution was centrifuged and other portion of the solution was filtered through 0.45 µm PVDF and 0.45 µm Nylon filters.

Table: 1.11 Results for filter validation of Dasatinib

S.No.	Filter details	Area Response	% Assay	Difference when compared to Centrifuged
1	Centrifuged Sample	10732682	104.5	NA
2	0.45 µm PVDF Filtered Sample	10718083	104.3	0.2
3	0.45 µm Nylon Filtered Sample	10728982	104.4	0.1

5.0 Results & Discussion

An RP-HPLC method for estimation of Dasatinib was developed and validated as per ICH guidelines. A simple, accurate and reproducible reverse phase HPLC method was developed for the estimation of Dasatinib in Dasatinib tablet formulations. The optimized method consists of mobile phase-A consisted of pH 5.8 phosphate buffer and Acetonitrile in the ratio of (90:10 v/v) and mobile phase-B consisted of Water and Acetonitrile in the ratio of (10:90 v/v) with Zorbax SB-C18 (150x4.6mm, 3.5µ) column. The retention time of Dasatinib was found to be 4.10 minutes. The developed method was validated as per ICH Q2A (R1) guideline.

We have developed a fast, simple and reliable analytical method for determination of Dasatinib in pharmaceutical preparation using RP-HPLC. As there is no interference of blank and placebo at the retention time of Dasatinib. It is very fast, with good reproducibility and good response.

The proposed HPLC method was linear over the range of 50.0789-150.2368 µg/mL, the correlation coefficient was found to be 0.9979. Relative standard deviation for method precision was found to be 1.3%.

The accuracy studies were shown as % recovery for Dasatinib 50%, 100% and 150% level. The limit of % recovered shown is in the range of 98 and 102% and the results obtained were found to be within the limits. Hence the method was found to be accurate.

The solution stability of standard and samples are stable upto 48 hours in refrigerator & on bench top conditions. For sample was stable up to 48 hours in refrigerator condition & stable up to 24 hours only on bench top condition.

Performed the filter validation for sample solution both 0.45 µm PVDF and 0.45 µm Nylon filters were compatible.

6.0 Conclusion

The developed method was validated for various parameters as per ICH guidelines like accuracy, precision, linearity, specificity, system suitability, solution stability and filter validation. The results obtained were within the acceptance criteria. The proposed method was applied for determination of Dasatinib in Dasatinib tablet formulation.

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Conflict of interests

The authors claim that there is no conflict of interest.

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