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RP-HPLC METHOD FOR ESTIMATION OF DASATINIB IN ACTIVE PHARMACEUTICAL INGREDIENT AND PHARMACEUTICAL DOSAGE FORM AS PER ICH GUIDELINES

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ABSTRACT

A simple, fast, accurate and precise UV-spectroscopic method and RP-HPLC method were developed and validated for the estimation of per ICH guidelines. The λ max of Dasatinib was found to be 315nm RP-HPLC method was developed by using Triethyl amine buffer solution PH 6.5 ±0.05 and solvent mixture (Methanol, Acetonitrile) in (50:50v/v) was used as the solvent and flow rate was set on 1.1 ml/min at 315 nm, retention time for Dasatinib was found to be 12 min. The method was developed in Cosmicsil BDS C18 column (100 mm × 4.6 mm, 3.5µm particle size). In RP-HPLC method was found to be linear in the range of is Dasatinib 50-150µg/ml and with a correlation coefficient value of 0.999. The accuracy studies of RP-HPLC method was performed at five different levels, i.e. 50%, 75%, 100%, 125% and 150% and recovery was found to be in the range of 101 to 101.5% for Dasatinib respectively. This method was Rugged and Robust in different testing criteria, The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be LOD value was 2.83 and LOQ value was 9.41 for RP-HPLC method. The % RSD is <2% which indicates the precision of the method. Results of all validation parameter were within the limit as per ICH guideline. So this method can be used for the determination of Bulk Drug as well as Tablet Dosage form easily and the method was precise, economical and accurate to perform in future.

KEYWORDS

Dasatinib, Methanol, Acetonitrile, UV, RP-HPLC and ICH.

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INTRODUCTION

Dasatinib is a Anti-Malarial Drug, molecular formula $C_{22}H_{26}Cl.N_7O_2S.H_2O$, IUPAC name BN-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyEthyl)-1-piperazinyl]-2-methyl4pyrimidinyl]amino] 5-thiazole Carboxamide monohydrate[1]. Dasatinib is a protein - tyrosine kinase inhibitor that inhibits the Bcr-Abi tytosine kinase, the constitutive abnormal tyrosine kinase create by the Philadelphia

chromosome abnormality in chronic myloid leukemia (CML). It inhibits proliferation and induces apoptosis in Bcr-Abi positive cells lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia[2].

According to literature review [3-10] there are very few method reported for the determination of Desatinib indifferent Instrumental techniques, out of these methods only 1 method were reported in Single Drug by using RP-HPLC.

Experimental Section

Standard drugs Dasatinib was procured from the Natco Pharma.ltd Chemicals and reagents. Methanol (Merck), Acetonitrile (Merck), Purified water (NA), Try ethyl amine (Merck), Ortho-phosphoric acid (Merck).

Instruments

HPLC Aliance waters (2487), UV Shimadzu Detector (UV detector) Dual absorbance detector, Column (BDS C18 column C18, (150 *4.6 mm, 5µ), Software (empower-2software), Sonicator (SV scientific).

Determination of absorption maxima by UV/Vis Spectrophotometers

Accurately weighed and transferred about 50mg of Dasatinib working standard into a 100ml volumetric flask, then added to it about 60 ml of methanol and sonicated for 10 minutes to dissolve and diluted up to mark with methanol and mix well. Further dilute 0.8ml of above solution to 10ml with methanol and mix well. Concentration of Dasatinib is about 8 ppm.

Dasatinib wavelength of detection was found by UV scan the above solution. The best possible wavelength was chosen as 315nm. The absorption maxima of dasatinib optimized by using UVspectrophotometer.

PREPARATION OF MOBILE PHASE

Buffer Preparation

4ml of triethylamine in 1000 ml milli-Q water and adjust PH6.5 ± 0.05 with Orthophosphoric acid. Then add 10 ml of methanol.

Solvent mixture

Prepare mixture of methanol and Acetonitrile in 50: 50 v/v respectively.

Mobile phase

Prepare a filtered (0.45µ*)* and degassed mixture of buffer preparation and solvent mixture in ratio of 50: 50 v/v respectively.

Diluent

Diluent-1 Solvent mixture

Diluent-11 Mobile phase.

Standard Preparation

Weigh and transfer accurately 50 mg of Dasatinib working Standard into a 100 ml clean dry volumetric flask, and add about 60 ml of solvent mixture sonicate to dissolve. Cool the solution to room temperature and dilute to volume with solvent mixture. Then transfer 2.0 ml above solution into 50 ml volumetric flask and dilute with mobile phase.

Sample preparation

Weigh and finely powder 20 tablets and Transfer the powder equivalent to 100 mg of Dasatinib into 250 ml of clean, dry, volumetric flask. Add about 160 ml of solvent mixture, shake on orbital shaker for 15 min and sonicated for 30 min with occasionally shaking. Cool the solution to room temperature and dilute volume with solvent mixture. Centrifuge the solution at 3000 RPM for 15 min. Then transfer 5.0 ml above solution into 100 ml volumetric flask and dilute with mobile phase.

Optimized chromatographic conditions

Column - Cosmicsil BDS C18 column (150*4.6mm), 5 µ

Flow rate - 1.0 ml/min

Wavelength - 315nm

Column temperature - 35˚C

Injection volume - 10 µl

Run time - 12 min

Method validation

The following parameters were considered for the analytical method validation of Dasatinib in pharmaceutical dosage form.

System Suitability

Chromatograph the standard preparations (6 replicate injections) and peak area responses for the analyte peak was measured and the system suitability parameters are evaluated. Tailing factor

for the peak due to Dasatinib in standard solution not more than 1.5. Theoretical plates for Dasatinib peaks in standard solution not less than 2500.

Accuracy

For accuracy determination, three different concentrations were prepared separately 50%, 75%, 100% and 125%, 150% for the analyte and chromatograms are recorded for the same.

Precision

The standard solution was injected for six times and the area was measured for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Robustness

As part of the Robustness, deliberate change in the temperature and flow rate Variation was made to evaluate the impact on the method.

Linearity and range

Linearity of the analytical method for assay by injecting the linearity solutions prepared in the range of 50µg to 150µg (10 ppm to 30ppm) of test concentration, into the chromatograph, covering minimum 6 different concentrations.

Ruggedness

Establish the ruggedness of the analytical method by using the assay of 6 different sample preparations of same batch by a different analyst using a different HPLC System

RESULTS AND DISCUSSION

Validation

Accuracy

Average recoveries of Lumefantrine are 100.6, 100.5, 100.8, 100.9, 100.0, at 50%, 75%, 100%, 25% and 150% concentrations level respectively. The percentage recoveries of the drug is within the limits 99-102%. So the method is Accurate. The accuracy study was performed for % recovery. The % recovery was found to be 101to 101.5% respectively. (NLT 98% and NMT 102%).

Precision

Precision are summarized in Table No.2 respectively. The % RSD values for Precession was less than 2.0%, which indicates that the proposed method is precise.

Linearity

The response was found linear over a concentration range of 100-500 µg/mL of Lumefantrine. The correlation co-efficient were found to be 0.999 for Lumefantrine. So the method is linear, data is presented in Table No.3. Linearity curve of Lumefantrine is given in Figure No.5.

Limit of Detection (LOD) and LOQ

The detection limit is determined by the analysis of samples with known concentration of analyte and by establishing that minimum level at which the analyte can reliably detected. The LOD are calculated from the calibration curve by formula LOD = 3.3 x SD/ b. The quantification limit is generally determined by the analysis of sample with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. The LOQ are calculated from the Calibration curve by formula LOQ = 10 x SD/ b.

Table No.1: Shows Accuracy Results of Dasatinib

Sample No.	Concentration Level	'mg/ml' added	'mg/ml' found	% Recovery	Average % Recovery
1	50%	0.0101	0.0101	100.00	100.6
2		0.0100	0.0102	101.67	
3		0.0100	0.0120	100.00	
4	75%	0.0152	0.0153	100.52	100.5
5		0.0152	0.0153	100.52	
6		0.0152	0.0153	100.52	
7	100%	0.0201	0.0203	100.83	100.8
8		0.0200	0.0202	100.83	
9		0.0201	0.0203	100.83	
10	125%	0.0253	0.0253	101.38	100.9
11		0.0250	0.0251	100.34	
12		0.0259	0.0252	101.04	
13	150%	0.0309	0.0303	101.11	101.0
14		0.0300	0.0303	100.83	
15		0.0300	0.0304	101.11	

The accuracy study was performed for % recovery. The % recovery was found to be 101to 101.5% respectively. (NLT 98% and NMT 102%).

Table No.2: Shows Precision Results of Dasatinib

S.No	Peak Name	Area
1	Dasatinib	775625
2	Dasatinib	776565
3	Dasatinib	774925
4	Dasatinib	777654
5	Dasatinib	775254
6	Dasatinib	774925
Mean	---	775824
SD	---	1084.32
% RSD	---	0.13

Table No.3: Shows Linearity Results of Dasatinib

Injection No	50% Level Solution	150% Level Solution
1	395125	1186797
2	395432	1186432
3	395243	1185643
4	396543	1187654
5	395215	1186754
6	395432	1186498
Average	395495	1186479
SD	2328.7	2152.36
% RSD	0.68	0.65

Table No.4: Shows Robustness Results of Dasatinib (Change in Flow Rate)

Replicate Standard injections at 0.9ml\min			
S.No	**Peak area**	**Observation**	**Acceptance criteria**
1	776292	Average :775705 % RSD = 0.10	% RSD : not more than 2%
2	774529		
3	775289		

Replicate Standard injections at 1.1ml\min			
S.No	**Peak area**	**Observation**	**Acceptance criteria**
1	776259	Average :775069 % RSD = 0.13	% RSD : not more than 2%
2	774425		
3	774525		

Table No.5: Shows Robustness Results of Dasatinib (Change in Temperature)

Replicate Standard injections at 32^0 C			
S.No	**Peak area**	**Observation**	**Acceptance criteria**
1	775289	Average :775777 % RSD = 0.433	% RSD : not more than 1%
2	775925		
3	776118		

Replicate Standard injections at 37^0 C			
S.No	**Peak area**	**Observation**	**Acceptance criteria**
1	776652	Average :776363 % RSD = 0.31	% RSD : not more than 1%
2	775325		
3	776112		

Table No.6: Shows Ruggedness Results of Dasatinib

S.No	Peak Name	Area
1	Dasatinib	775269
2	Dasatinib	774215
3	Dasatinib	776152
4	Dasatinib	774952
5	Dasatinib	775962
6	Dasatinib	776119
Mean	---	775445
SD	---	775.65
% RSD	---	0.1002

Table No.7: Shows LOD and LOQ Results of Dasatinib

S.No	Parameters	Dasatinib
1	LOD	2.83µg/ml
2	LOQ	9.41µg/ml

Table No.8: Shows Summary of Validation Parameter Results

S.No	Parameter	Acceptance criteria	Results Obtained
1	% recovery	98-102%	101
2	Linearity range(µg/ml)	---	50-150 µg/ml
3	Correlation coefficient	NLT 0.999	0.999
4	No.of Theoretical plates	NLT 2500	3255
5	Precision % RSD	% RSD (NMT 2%)	0.13
6	Intermediate Precision % RSD	% RSD (NMT 2%)	0.1002
7	LOD	-	2.83µg/ml
8	LOQ	-	9.41µg/ml

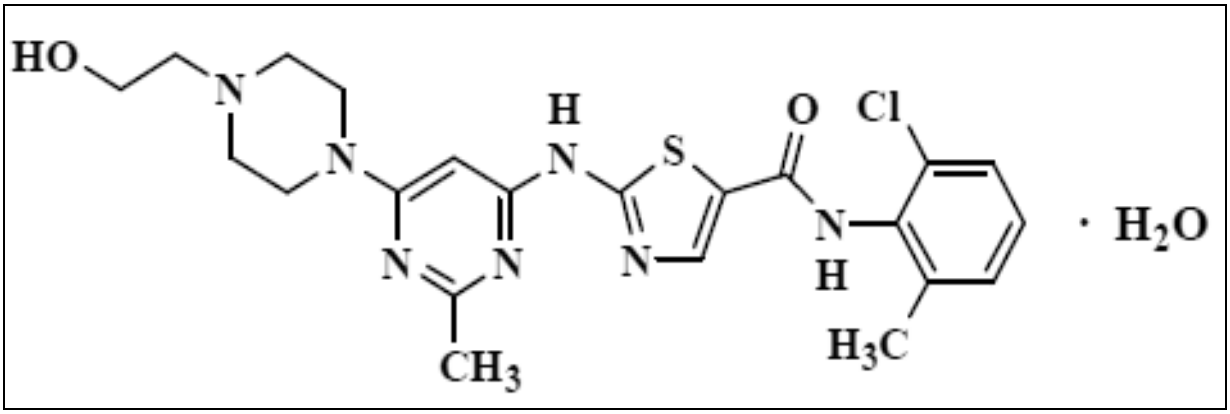

Figure No.1: Shows Structure of Dasatinib Monohydrate

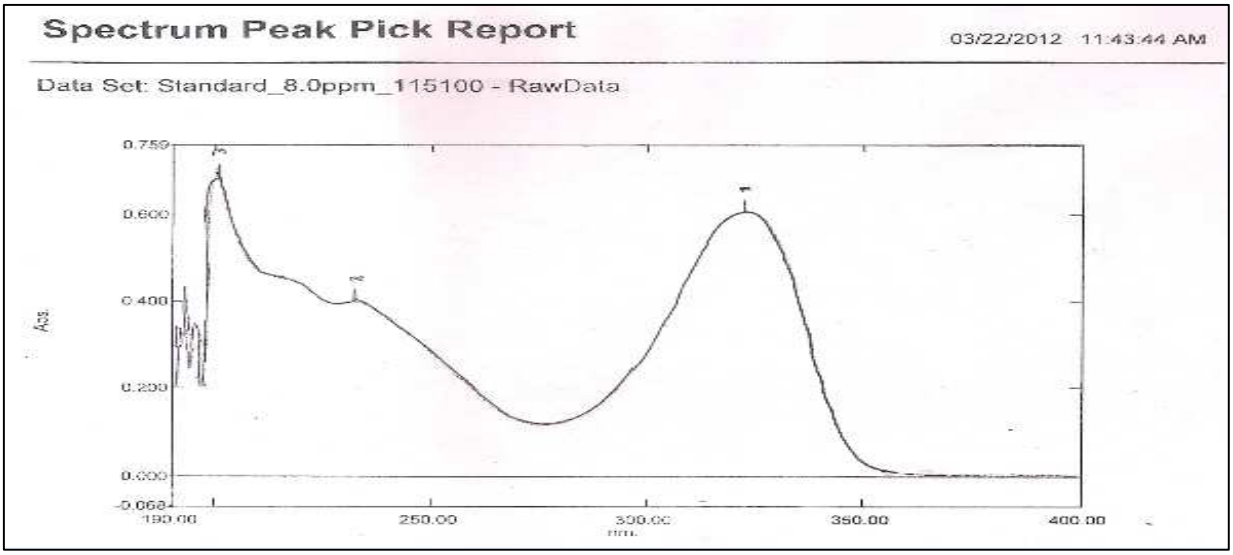

Figure No.2: Shows UV Spectrum of Dasatinib

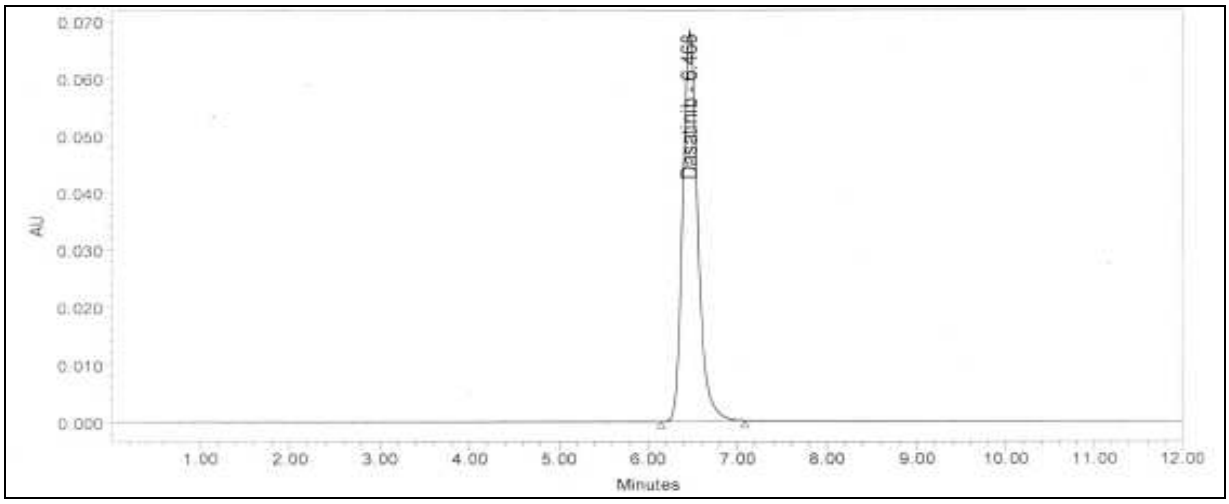

Figure No.3: Shows Standard Chromatogram of Dasatinib

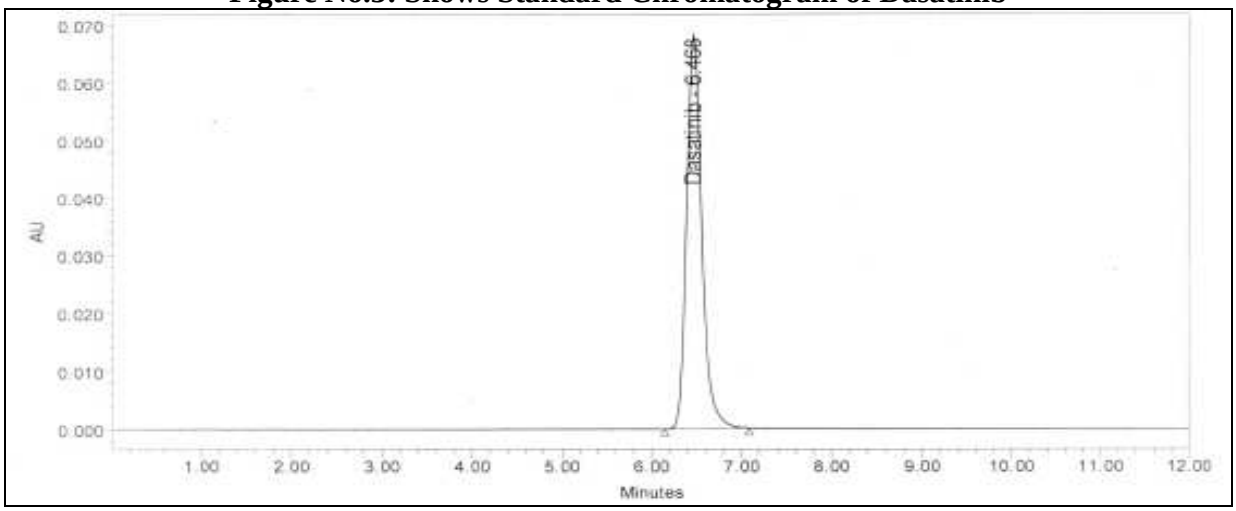

Figure No.4: Shows Sample Chromatogram of Dasatinib

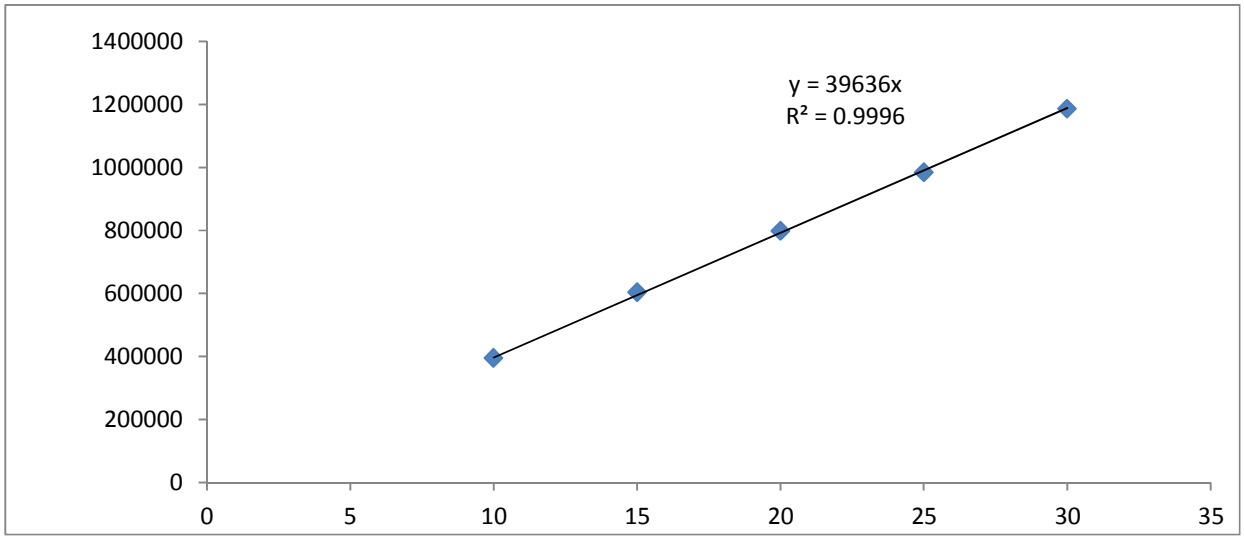

Figure No.5: Shows Calibration Graph of Dasatinib

CONCLUSION

Method development and validation of Dasatinib was done by RP-HPLC method. The estimation was done byusing Cosmicsil BDS C18 column (150*4.6mm) 5 µ Make Analytical technologies) mobile phase as Triethyl amine buffer solution pH 6.5 ±0.05 and solvent mixture (Methanol, Acetonitrile) in (50:50v/v) was used as the at a flow rate 1.1ml/min. The linearity range of Dasatinib was found to be 50-150 µg/ml. Correlation coefficient value was 0.999, values of % RSD was 0.13 which is within the limit. These results show the method is accurate, precise, sensitive, economic and rugged. The HPLC method is more rapid. The proposed method is successfully applied to the bulk dosage form. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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