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Research Article

Advanced Eco-Friendly Novel RP-HPLC Method for Estimation of Dasatinib in Tablets by Qbd: Greenness Assessment through GAPI and AGREE

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ABSTRACT

Introduction: An advanced Eco-friendly novel chromatographic method was developed and validated for the estimation of Dasatinib in tablet dosage form by Quality by design approach. Design of experiments was applied for the optimization for RP-HPLC method and greenness of the method was evaluated. ***Materials and Methods:*** Preliminary screening trials along with systemic risk analysis were performed, endeavouring to explicate the critical method attributes (CMAs), namely Amount of organic modifier, Flow rate and pH of buffer that influence critical quality attributes (CQAs). Box-behnken design was employed to study the response surface methodology for analyzing the interaction of each three level on Retention time(R1) and Tailing factor(R2) were considered as response factors. The RP-HPLC method of Dasatinib was successfully developed and validated according to ICH Q2(R1) guidelines with respect to linearity, accuracy, precision, robustness, Limit of Detection and Limit of Quantitation. The greenness scale of developed method using the "analytical GREENness (AGREE)" and "Green Analytical Procedure Index (GAPI)" approach. ***Results and conclusion:*** The regression data for the calibration plots exhibits linear relationship over a concentration range 5-25 µg/ml. The % RSD for both inter- day and intra- day precision was less than 2%. Percent recovery of Dasatinib was found to be more than 99%. The assay was found to be 99.95 ± 0.23%. AGREE and GAPI was applied to proposed analytical method and showed an excellent greenness characteristic of the present approach. The developed eco-friendly method was accurate, precise, and cost effective. Developed greener chromatographic method can be used for routine analysis of Dasatinib.

INTRODUCTION

Dasatinib [N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl] amino]-5-thiazolecarboxamide monohydrate] is an oral anticancer medication, which belongs to tyrosine kinase inhibitor used for treating chronic myeloid leukaemia and acute lymphoblastic leukaemia. The empirical formula and Mol. wt. are $C_{22}H_{26}ClN_7O_2S$ and 488.01 g/mol respectively. It is white to off- white powder, melts at 280- 286 ºC. It is slightly soluble in Methanol, Ethanol, Acetone and Acetonitrile; very poorly soluble in water [1, 2]. Chemical structure of Dasatinib is in Figure 1.

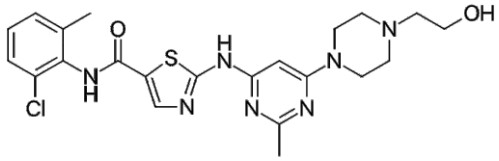

Figure 1: Structure of Dasatinib

AQbD (Analytical Quality by Design) is a systematic, risk-based and proactive approach to analytical method development which focuses on identifying and minimizing sources of variability and failure modes that may lead to establish robust method operable design space within meaningful system suitability criteria and ensuring that the method meets its intended performance requirements throughout the product and method lifecycle [2-9].

Green chemistry principles have recently attracted scientist's interest to reduce human health and environmental risks. Various criteria are currently utilized to evaluate the environment impact of various analytical methodologies. The analytical GREENness (AGREE) calculator is a Panoramic, adaptable and clear approach to assessing the greenness of analytical procedures which is based on 12 green chemistry principles [10-14]. The green analytical procedure index (GAPI) tool used to evaluate the green nature of developed procedures. The GAPI metric uses a pictogram to classify the greenness of each ste-

p of an analytical methodology, applying a colour scale, with two or three levels of evaluation for each stage [1-5].

Using AQbD concept, we were able to optimize and develop novel RP- HPLC method for the estimation of Dasatinib, which is efficient and cost effective. Further, the optimized method was validated according to ICH Q2 (R1) regulations [16, 17], and was successfully applied to the quantification of Dasatinib.

Detailed Review of Literature survey revealed that reported conventional methods in bulk and pharmaceutical formulation which include, UV Spectrophotometric methods [18] and RP- HPLC [19-22], were found to be more time consuming and expensive.

Furthermore, no greenness profile was estimated in the above reported analytical methods. HPLC method optimization of dasatinib by box-behnken design approach was not studied and reported in the literature. Based on above facts and observations, the goal of the present study is to design and evaluate eco- friendly RP- HPLC method for the detection of Dasatinib in marketed tablet formulation.

MATERIAL AND METHODS

Chemicals and Reagents

Reference standard sample of Dasatinib was procured from Apex Pharma Chem, Ankleshwar. Marketed formulation of Dasatinib Tablets 20 mg (Dasanat 20), marketed by Natco Pharma, Purchased from Local Pharmacy store. HPLC grade Water, Methanol and Acetonitrile were purchased from Merck Specialties Pvt. Ltd. Analytical grade Ammonium acetate, Potassium di-hydrogen orthophosphate, Hydrochloric acid, Potassium hydroxide and Glacial acetic acid were purchased from Finar Chemical Ltd.

Instrumentation

The chromatographic method was developed and validated on Shimadzu LC- 10 AT (Shimadzu Corporation, Kyoto, Japan). This HPLC system consists of UV detector and 20µL fixed loop injector. Analytical chromatographic data were collected and processed using Lab solution software. Selected Mobile phase was degassed using Frontline electronics- FS 5 ultra sonicator. Absorbance spectra were recorded using a UV-VIS spectrophotometer.

Software(s)

For Experimental design, Data analysis (ANOVA) and desirability function estimate were performed by Design Expert® version 12.0. Method greenness assessment was performed by AGREE: The analytical greenness calculator (version 0.5, Gdansk University of Technology, Gdansk, Poland, 2020) and ComplexGAPI (version 02-beta).

Preparation of standard solution

Accurately weighed dasatinib standard (10 mg) was transferred into 10 ml volumetric flask, and dissolved in and diluted to the mark with 10 ml with methanol to obtain 1000 µg/ml standard stock solution. The stock solution was further diluted by 1 ml of stock solution to 10 ml with methanol to get to a sub-stock 100 µg/ml. This sub-stock solution was serially diluted with mobile phase to obtain solutions in the linearity range of (5-25 µg/ml).

Selection of Detection Wavelength

In the present study, standard solution of dasatinib (10 µg/ml) prepared in methanol was scanned in UV region of 200-400 nm for the selection of the detection wavelength.

Method development by using the QbD concept

Step 1: Analytical target profile (ATP)

The first step was to define the objectives of the method development, called ATP. The main objectives were to optimize chromatographic condition to improve the quality of chromatogram. Secondly, successfully apply the developed method for estimation of dasatinib.

Step 2: Determine quality target product profile (QTPP)

The QTPP plays an important role for identifying the variables that affect the chromatographic conditions. The retention time and tailing factor were identified as QTPP (Dependent variables) for proposed RP- HPLC method development.

Step 3: Determine critical quality attributes (CQA)

The CQAs are the method parameters that are directly affect the QTPP. Three critical method parameters were considered for proposed RP- HPLC method development, having considerable effect on retention time and tailing factor are % organic modifier, Flow rate and pH of buffer.

Step 4: Selection of Levels and performing experimental design

Usually, the factors were examined at three levels (-1, 0, +1) or more in optimization experimental designs as per **Table 1**. For method optimization, the range between the levels of a factor is chosen on the basis of earlier gathered knowledge and/or literature information. After defining the QTPP and CQAs, Box- behnken design (BBD) was applied to optimization. BBD could predict optimum condition with lowest runs and therefore saving time and cost, considered for study.

Step 5: Analysis of experimental results and optimization of the method

Systemic statistical analysis of experimental results was carried out using Design Expert® (Version 12.0). Statistical

tools like predicted vs. actual plot, ANOVA, lack of fit, prediction equations and contour plots were used to analyze each individual response parameter and design space was generated.

Chromatographic conditions

The Chromatographic separation was carried out in isocratic mode using a Mobile phase consisting of a mixture of Methanol: Ammonium acetate buffer, pH 3.5 adjusted with 2M HCl in the ratio of 60:40 at a flow rate of 1.0 ml/min. The eluted drug was detected at 270 nm with UV detector. The sample injection volume was 20 µl. The HPLC system was maintained at temperature $30 \pm 2^{\circ}C$.

Validation of developed chromatographic method

The developed HPLC method for estimation of Dasatinib was validated as per ICH Q2 (R1) guidelines [11].

Specificity

Specificity of method was established by the peak purity study. Peak purity values were obtained to be more than 0.998. Peak Profiling values indicating that there are non-interference of any other peak of degradation product or impurities.

Linearity and Range

Linearity was assessed by analyzing standard solution in a range of 5- 25 µg/ml Dasatinib. Standard Calibration curve was plotted and Correlation coefficient (r^2) was found.

System Precision

Repeatability

For RP- HPLC analysis, six replicate injections of samples were analyzed over a short period of time. Repeatability is also termed as intra- assay precision. Repeatability study carried out using Dasatinib solution containing 15 µg/ml concentrations. Then average peak area and % RSD were calculated.

Intermediate Precision

The Intermediate Precision of analytical method demonstrated by Intraday and Interday Precision. In Intraday Precision, Standard solution containing Dasatinib (10, 15, 20 µg/ml) were analyzed three times in same day (0 hr, 3 hr and 6 hr) and then average peak area and % RSD were calculated. In Interday Precision, Standard solution containing Dasatinib (10, 15, 20 µg/ml) were analyzed in three different days (Day- 1, 2 and 3) and then average peak area and % RSD were calculated.

Accuracy (Recovery study)

To measure accuracy of analytical method, recovery studies were carried out using standard addition method with differe--nt level 80 %, 100 % and 120 %. The results of recovery studies indicated that the method is accurate for the estimation of Dasatinib.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection is a lowest concentration in a sample that can be detected but not necessarily quantified under the optimized experimental conditions. The Limit of Quantitation is lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

Robustness

The robustness of method was determined under variable conditions. The robustness of developed analytical method was established by illustrating its reality against consider changes in the optimized chromatographic conditions.

Assay

Twenty tablets (each containing 20 mg Dasatinib) were accurately weighed and average weight was calculated. The tablets were grinded and mixed well. An accurately weighed powder equivalent to 20 mg of Dasatinib was transferred to 100 ml volumetric flask. Add 60 ml of methanol, and then solution was sonicated for 20 min until the powder dissolves. Then, make up the volume upto mark with same solvent. Filter the resulting solution with 0.42 µ Whatman filter paper. An aliquot (0.5 ml) was transferred into 10 ml volumetric flask and diluted with mobile phase to obtain a sample solution of 10 µg/ml. Then the solution was analyzed using the proposed Chromatographic method.

Greenness assessment

Using AGREE: The analytical Greenness calculator, the greenness assessment for developed and validated analytical assay method for Dasatinib was evaluated. The assessment criteria are based on the 12 significant principles, and alternative weights can be applied to them, allowing for some flexibility. Each one of the 12 input variables is converted to a common scale ranging from 0 to 1.

The GAPI represents total 15 pictograms, each representing a step within the main 5 pentagrams; each pentagram shows the greenness of each step of an analytical methodology. Each step represented by three levels of colour scale as per greenness index. In GAPI, reagents, procedures, and instrumentation are evaluated.

RESULT & DISCUSSION

Method development

In this experimental work, maximum absorbance was recor-

-ded at 270 nm as in **Figure 2**. Therefore, it was selected as detection wavelength for further analysis.

For mobile phase optimization various solvent combinations have been tried using water and acetate buffer with different organic modifiers like acetonitrile and methanol. For method optimization, the range between the levels of independent variables is chosen on the basis of earlier gathered knowledge and/or literature information as (**Table 1**).

Box- behnken design values of the 15 experimental runs and their results are shown in **Table 2 and 3**. The collected data were subjected to statistical analyses using Design- Expert® software version 12.

A steepest slope or curvature indicates the sensitiveness of the response to a particular factor. From Fig. 3(A) and 3(C) and **equation retention time (for actual values) = 110.988 + 2.416 (A) - 39.061 (B) - 0.078 (C) + 0.366 (A × B) - 0.051 (A × C) -1.050 (B × C) + 0.016 (A^2) + 7.498 (B^2) + 0.545 (C^2)**, it was concluded that as β_1 positive coefficient (2.416) suggests that as the amount of methanol in the mobile phase (A) increases, β_2 negative coefficient (-39.061) suggests that as flow rate (B) decreases and β_3 negative coefficient (-0.078) suggests that as pH of buffer (C) decreases, the value of retention time was increased.

From Figure 3(B) and 3(D) and **equation tailing factor (for actual values) = 22.974 + 0.267 (A) – 1.807 (B) + 7.030 (C) – 0.027 (A × B) – 0.005 (A × C) + 0.262 (B × C) + 0.0025 (A^2) + 1.298 (B^2) + 0.982 (C^2)**, it was concluded that as β_1 positive coefficient (0.267) suggests that as the amount of methanol in the mobile phase (A) increases, β_2 negative coefficient (-1.807) suggests that as flow rate (B) decreases and β_3 positive coefficient (7.030) suggests that as pH of buffer (C) decreases, the value of tailing factor was increased.

Method optimization

Optimization was obtained by studying all responses in different experimental conditions using the Design expert® 12.0 software, and optimized HPLC conditions and predicted responses are shown below. Overlay plot of optimized condition is shown in **Figure 4**.

Obtained solution for optimized condition

A. % Organic Modifier: 60.00
B. Flow rate: 1.0
C. pH of buffer: 3.5

t_R DASA: 6.686
Tf DASA: 1.242

The observed value for responses was calculated by running the HPLC chromatogram for given set of amount of methanol, Flow rate and pH of buffer and then compared with the predicted values to evaluate for % predicted error.

Method Validation

Linearity and Range

Calibration curve for Dasatinib was linear over the concentration range of 5- 25 µg/ml shown in **Figure 5** and **Table 4**. The regression equation for the calibration curve was found to be y = 42,972.030x – 5009.35 with a 0.999 correlation coefficient (R^2) when standard calibration curve was plotted with peak area verses concentration (**Figure 6**).

System Precision (Repeatability)

Repeatability was assessed by using six replicates of standard solution of Dasatinib containing 15 µg/ml concentrations. Then % RSD was found to be 0.227 (**Table 5**).

Intermediate Precision

The Intermediate precision demonstrated by Intraday and Interday Precision. Interday and Intraday precisions were shown in **Table 5**. The % RSD value less than 2 indicated that the developed method was found to be precise.

Accuracy (Standard addition method)

Recovery studies were performed with previously analysed samples of Dasatinib (10 µg/ml) were spiked with 80 %, 100 % and 120 % Dasatinib standard and the mixtures were analysed by the proposed method. The amount of Dasatinib was calculated (**Table 6**) and % recovery found satisfactory.

LOD and LOQ

The LOD and LOQ for dasatinib based on standard deviation of slope and intercept were found to be 0.0126 µg/ml and 0.0382 µg/ml.

Robustness

The robustness of method carried out using 15 µg/ml solution of Dasatinib. The % RSD for peak area were found to be less than 2 by change in pH (± 0.5), flow rate (± 0.1 ml/min) and amount of organic modifier (± 5 ml) demonstrated in **Table 7**.

Assay

In Assay, Mean (n=5) mg of Dasatinib found to be 19.99 mg. Assay of Dasatinib Tablets were found to be 99.95% ± 0.235.

Greenness Assessment

Two assessment tools were used for ensuring the greenness of analytical method including AGREE and GAPI. AGREE considers all twelve green chemistry principles to derive the greenness profile. The sum of all each twelve criteria is the final assessment result. The performance of the procedure in each of the assessment criteria is reflected by the colour in the segment with the number corresponding to each criterion. The scores corresponding to GAC principles 7, 9 and 10 are quite low, due to generation of large volume of analytical waste, energy used and type of reagents used during analysis,

respectively. While, in the case of principles 2 (minimum sample size), 3 (type of measurement), 4 (Integration of analytical processes), 5 (Automated and miniaturized methods) and 11 (Toxic reagents should be eliminated or replaced), excellent greenness score was achieved. A middle colour of AGREE pictogram is somewhat light green with a score of 0.69, indicating that the developed method has a low environmental impact and can be regarded as a green method . In accordance with GAPI, each analytical assessment was started with sample preparation. Pictogram created for GAPI utilizes a colour scale, with two or three levels of evaluation for each stage. The created pictogram can be used to evaluate and quantify from green to yellow to red colour respectively for the low, medium and high environmental impacts associated with each stage of the pre-analysis process and the analytical methodology. GAPI pictogram showing only three red zones which corresponding to physico-chemical analysis, non- greener solvent used and no waste treatment. A representative diagram of pictogram for AGREE score and GAPI score of the proposed analytical assay is shown in **Figure 7**.

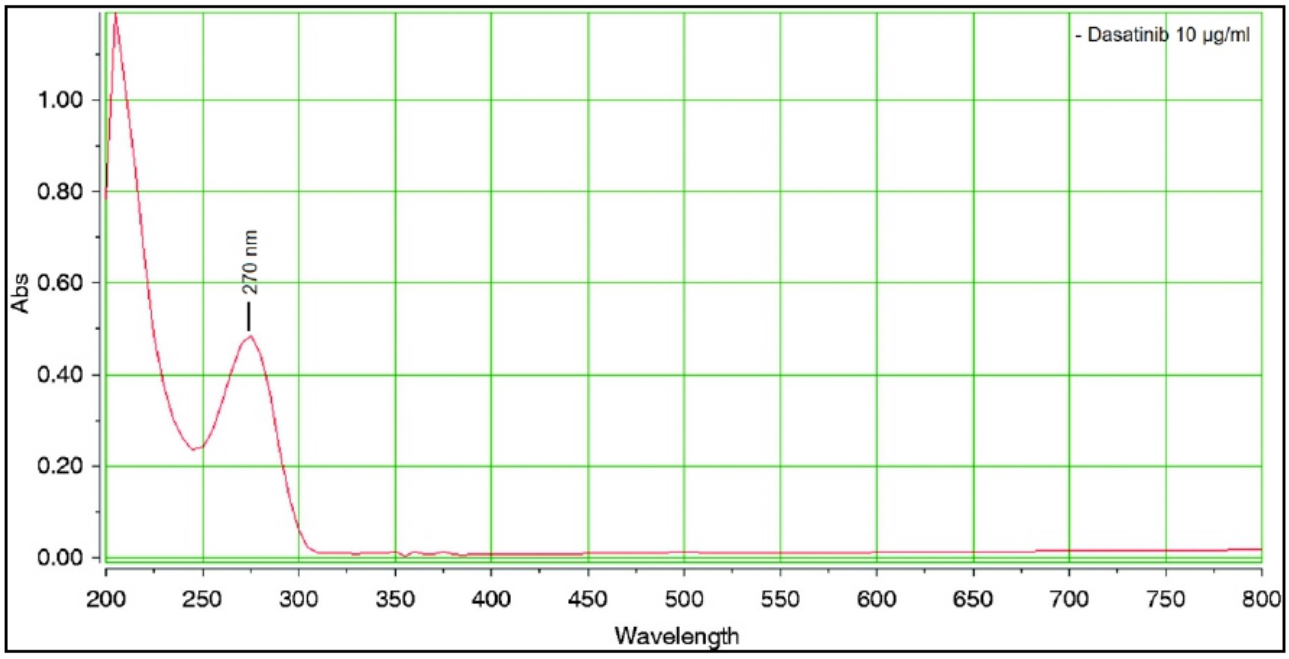

Figure 2: UV Spectra of Dasatinib (10 µg/ml) (Detected Wavelength 270 nm)

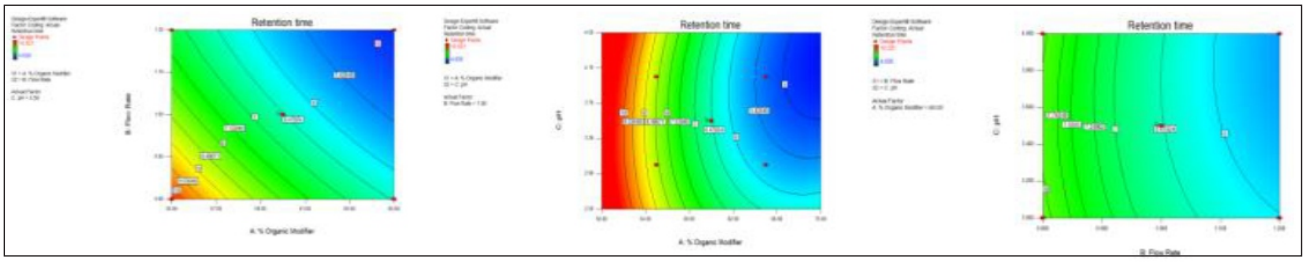

Figure 3A: Contour plots showing effect on t_R of (a) Factor A and B; (b) Factor A and C; (c) Factor B and C

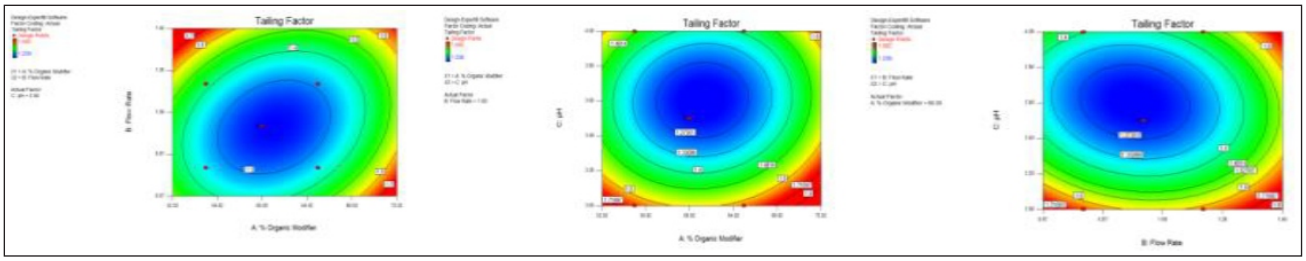

Figure 3B. Contour plots showing effect on Tf of (a) Factor A and B; (b) Factor A and C; (c) Factor B and C

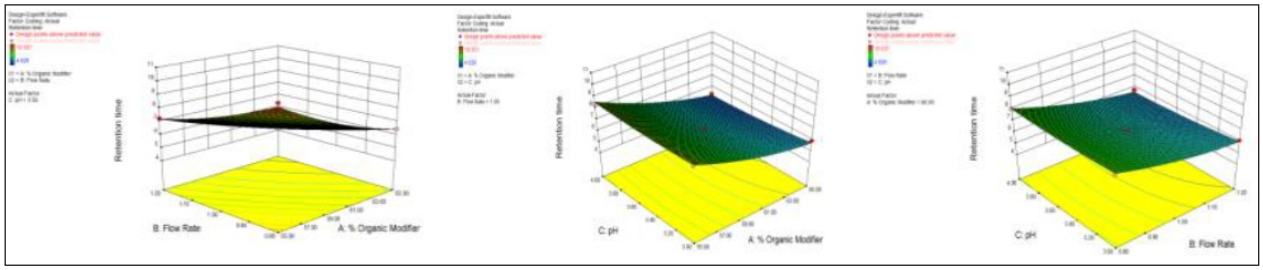

Figure 3C: 3D plots showing effect on t_R of (a) Factor A and B; (b) Factor A and C; (c) Factor B and C

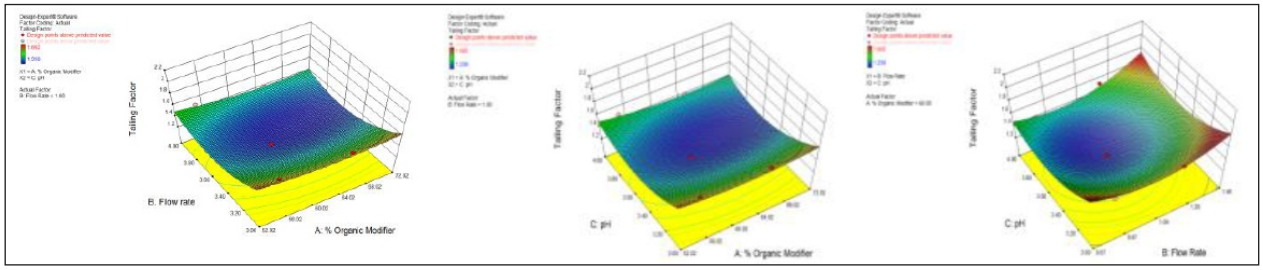

Figure 3D: 3D plots showing effect on Tf of (a) Factor A and B; (b) Factor A and C; (c) Factor B and C

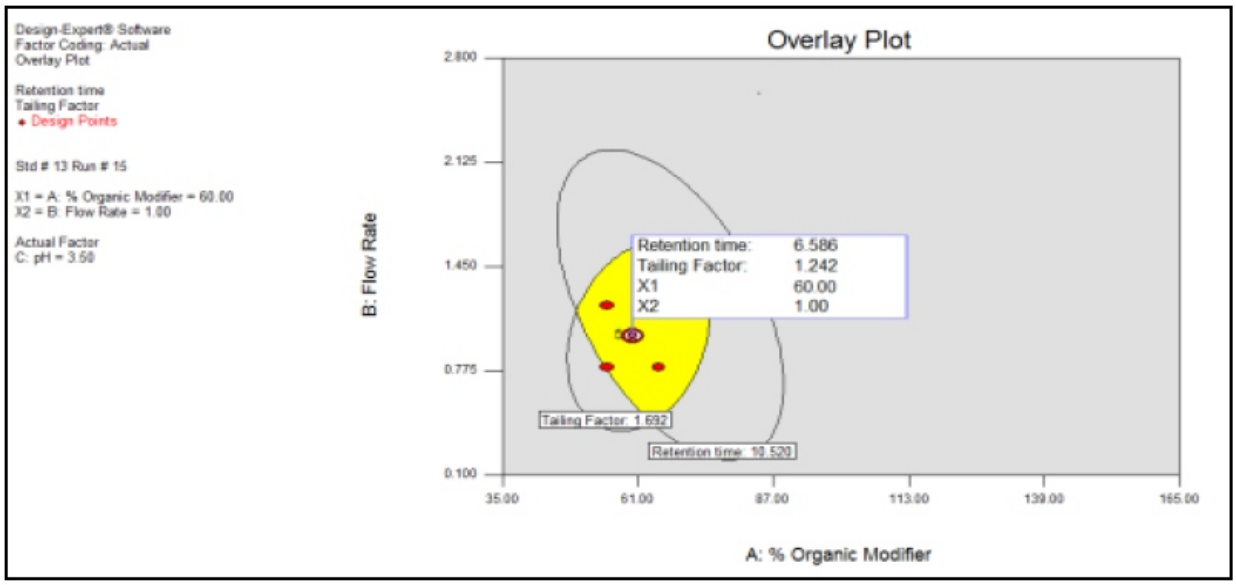

Figure 4: Overlay plot of optimized condition

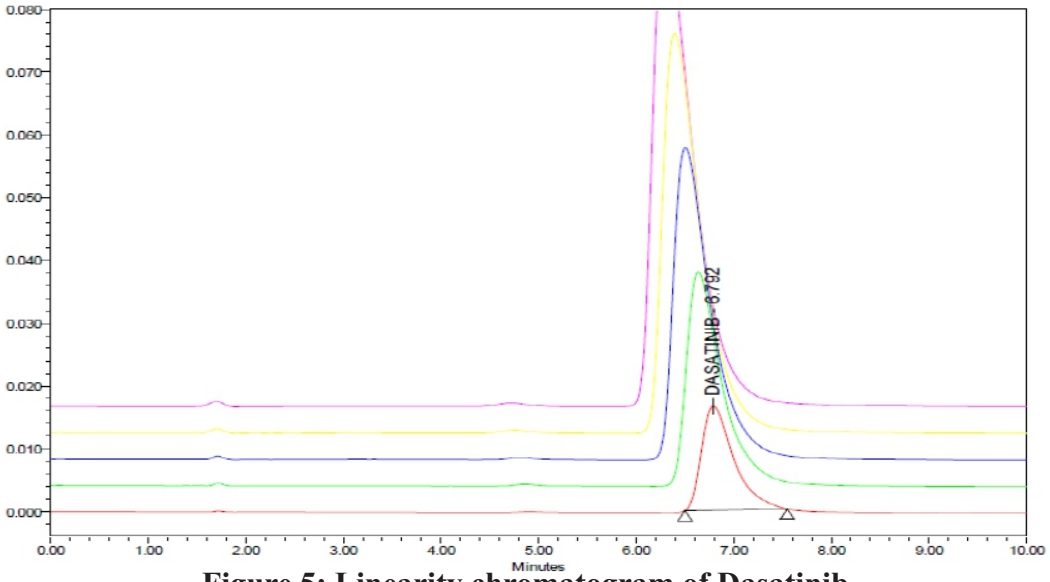

Figure 5: Linearity chromatogram of Dasatinib

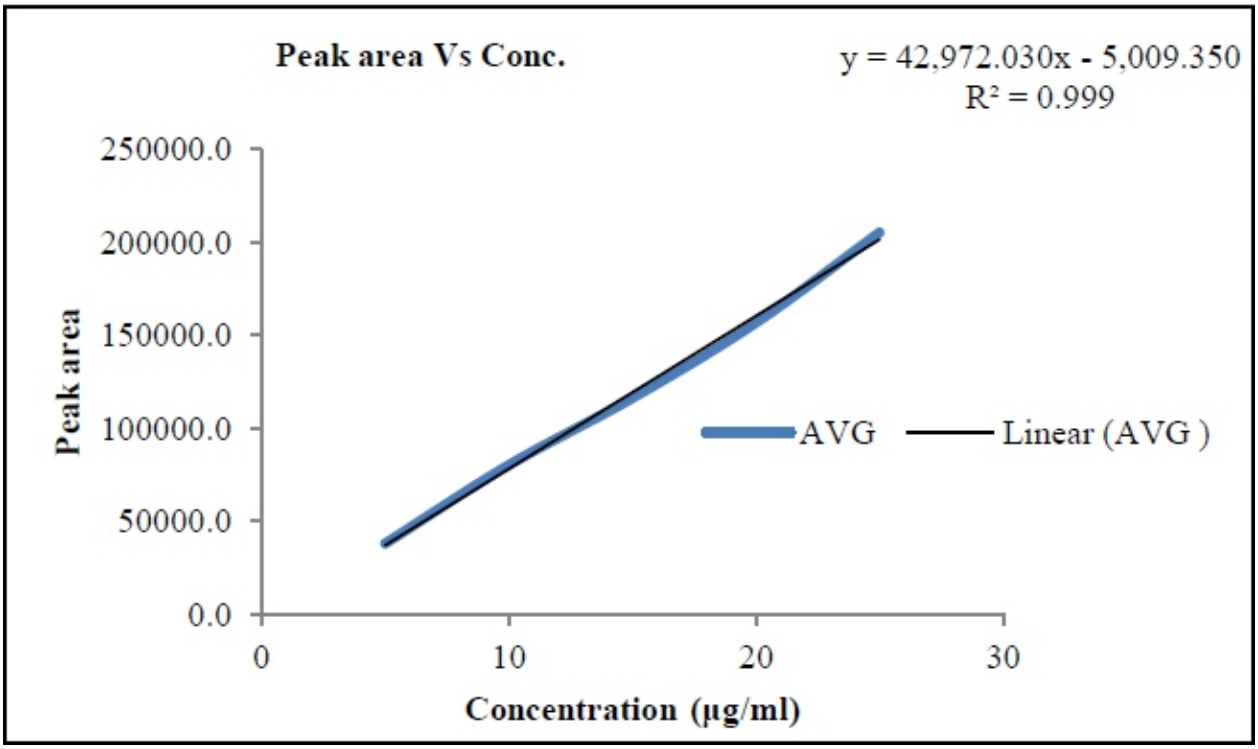

Figure 6: Standard calibration curve of Dasatinib (5-25 µg/ml)

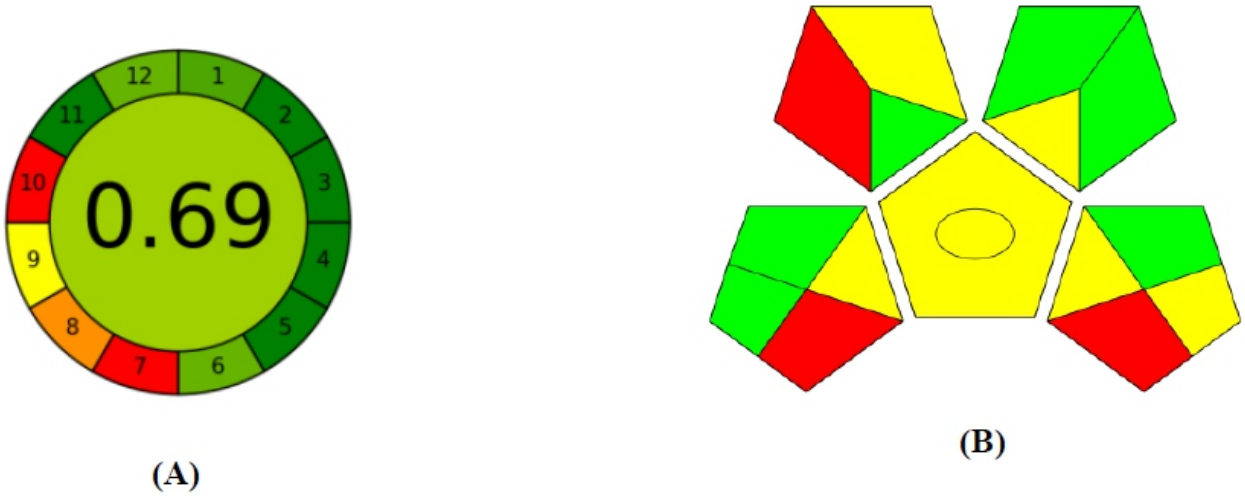

Figure 7: The representative pictogram for (A) AGREE score (B) GAPI score of the proposed HPLC method

Table 1: Levels of independent variables

Concentration of Factor	Lower level (-1)	Intermediate level (0)	Higher level (+1)
% organic modifier (A)	55	60	65
Flow rate (B)	0.8	1.0	1.2
pH of buffer (C)	3.0	3.5	4.0

Table 2: Box- behnken design with measured response

Run	Pattern (X1X2X3)	Factor A. % Organic modifier	Factor B. Flow rate (ml/min)	Factor C. pH	Response - 1 Retention time t_R (min)	Response - 2 Tailing factor Tf
1	(+1,0,-1)	65	1	3	5.931	1.647
2	(0,0,0)	60	1	3.5	6.507	1.24
3	(+1,-1,0)	65	0.8	3.5	6.428	1.382
4	(0,-1,-1)	60	0.8	3	7.961	1.614
5	(0,-1,+1)	60	0.8	4	7.989	1.397
6	(0,0,0)	60	1	3.5	6.509	1.248
7	(+1,0,+1)	65	1	4	5.336	1.385
8	(-1,+1,0)	55	1.2	3.5	7.253	1.389
9	(-1,-1,0)	55	0.8	3.5	10.521	1.366
10	(0,+1,-1)	60	1.2	3	6.107	1.629
11	(0,+1,+1)	60	1.2	4	5.715	1.517
12	(-1,0,+1)	55	1	4	8.415	1.482
13	(0,0,0)	60	1	3.5	6.504	1.239
14	(-1,0,-1)	55	1	3	8.492	1.692
15	(+1,+1,0)	65	1.2	3.5	4.626	1.294

t_R= Retention time, Tf= Tailing Factor

Table 3: ANOVA of reduced model for response t_R and Tf

Source	Sum of Squares		Df		Mean square		F value		P value	
	t_R	Tf	t_R	Tf	t_R	Tf	t_R	Tf	t_R	Tf
Model	31.69	0.39	9	9	3.52	0.044	91.78	37.71	< **0.0001**	< **0.0001**
A- % Org. modifier	19.10	0.006105	1	1	19.10	0.006105	497.68	5.27	< **0.0001**	0.0553
B- Flow rate	10.58	0.0006125	1	1	10.58	0.0006125	275.61	0.53	< **0.0001**	0.4906
C- pH	0.13	0.080	1	1	0.13	0.080	3.50	69.26	0.1037	< **0.0001**
AB	0.54	0.003080	1	1	0.54	0.003080	14.00	2.66	**0.0072**	0.1469
BC	0.067	0.0006760	1	1	0.067	0.0006760	1.75	0.58	0.2277	0.4698
AC	0.044	0.002756	1	1	0.044	0.002756	1.15	2.38	0.3193	0.1668
A^2	0.68	0.017	1	1	0.68	0.017	17.60	14.99	< **0.0001**	**0.0061**
B^2	0.38	0.011	1	1	0.38	0.011	9.87	9.81	**0.0163**	**0.0165**
C^2	0.078	0.25	1	1	0.078	0.25	2.04	219.52	0.1960	< **0.0001**
Residual	0.27	0.008105	7	7	0.038	0.001158				
Lack of fit	0.27	0.008052	3	3	0.090	0.002684	2712.914	201.81	< **0.0001**	< **0.0001**
Pure Error	0.00001320	0.00005320	4	4	0.0000033	0.00001330				
Cor Total	31.96	0.40	16	16						

Table 4: Linearity of Dasatinib (5 – 25 µg/ml)

Concentration (µg/ml)	Peak Area (Mean ± SD) (n=6)
5	37987.50 ± 69.87
10	80161.33 ± 99.67
15	116194.00 ± 247.18
20	156691.16 ± 260.06
25	205194.83 ± 464.29

Table 5: Precision data for Dasatinib

Repeatability			
Conc. (µg/ml)	Peak area	Mean (n= 6) ± SD	% RSD
15	116109	116199 ± 264.59	0.227
	116714		
	116124		
	116085		
	115958		
	116204		
Intraday and Interday			
Conc. (µg/ml)	Peak area (Mean, n= 3) ± SD, % RSD	Peak area (Mean, n= 3) ± SD, % RSD	
10	80108.66 ± 88.58, 0.110	80117.33 ± 100.75, 0.125	
15	116250.33 ± 236.747, 0.203	116261.00 ± 329.56, 0.283	
20	156534.00 ± 349.39, 0.223	156569.33 ± 474.77, 0.303	

Table 6: Accuracy of Dasatinib

Conc. level	Amt. taken	Amt. added	Amt. recovered	% Amt. found ± SD	% RSD
	µg/ml				
80 %	10	8	8.04	100.56 ± 0.622	0.619
100 %	10	10	9.91	99.18 ± 0.190	0.192
120 %	10	12	12.11	100.95 ± 0.177	0.175

Table 7: Robustness data for Dasatinib

Parameters	Normal conditions	Normal Rt	Deliberate changes	Retention time in min. (Rt) (Mean, n=6) ± SD, %RSD
Flow rate	1.0 ml/min	Dasatinib- 6.507 min.	1.1 ml/min	6.493 ± 0.0085, 0.0085
			0.9 ml/min	6.513 ± 0.0075, 0.0075
pH of Mobile phase	3.5		4.0	6.476 ± 0.0105, 0.162
			3.0	6.508 ± 0.0154, 0.236
Amt. of organic modifier	60 ml		65 ml	6.497 ± 0.0225, 0.346
			55 ml	6.521 ± 0.0110, 0.168

CONCLUSION

Analytical quality-by-design approach to RP-HPLC method development has been described. The method was successfully developed and optimized through DOE, and data were analyzed using Design Expert® version 12.0 software. The significant effect of independent factors was analysed using ANOVA, and the effect was also reported in form of perturbation plots. DOE provides efficient tools for the optimization of variable factors for RP-HPLC method development. The HPLC method was successfully validated as per ICH guidelines. All validation parameters were found within the acceptance criteria. Proposed analytical method was successfully applied for greenness assessment using AGREE and GAPI tools and method was found to be greener. As per results, the validated method is novel, sensitive, linear, accurate, precise, robust and eco- friendly for the analysis of Dasatinib in tablet dosage form. The QbD approach to method development has helped to better understand the method variables hence leading to less chance of failure during the method validation and it can be used for routine analysis of Dasatinib.

LIST OF ABBREVIATIONS

RP-HPLC: Reverse phase high performance liquid chromatography; **ANOVA:** Analysis of Variance; **AGREE:** Analytical Greenness; **GAPI:** Green Analytical Procedure Index; **µg/ml:** Microgram per ml; **ATP:** Analytical Target Profile; **CQA:** Critical Quality Attributes; **QTPP:** Quality target product profile; **ICH:** International Council for Harmonisation; **Rt:** Retention time; **Tf:** Tailing factor; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **RSD:** Relative standard deviation.

ETHICS APPROVAL

Not Applicable

AVAILABILITY OF DATA AND MATERIAL

Not Applicable

CONFLICT OF INTERESTS

Declared none

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AUTHOR'S CONTRIBUTION

The idea was developed by M.R.P. and H.U.P. The QbD optimization was carried out by M.R.P. and H.U.P. assisted with development and validation of analytical method. Both authors are contributed in collaborate and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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