



Research Article

CHEMOMETRICS ASSISTED RP-HPLC METHOD DEVELOPMENT FOR THE SEPARATION OF SECOND GENERATION TYROSINE KINASE INHIBITORS IN BULK DRUGS AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

The present study describes a newly developed, optimized and validated isocratic RP-HPLC method for the separation of two tyrosine kinase inhibitors (Dasatinib-DST and Nilotinib-NLT) with Methyl paraben-MPB as internal standard (IS), in bulk and pharmaceutical formulations with the aid of Chemometrics, multi criteria decision making (MCDM) approach. The separation was achieved by using Phenomenex Enable C18 column (15×4.6 mm id, 5µm particle size) and PDA-UV-detection at 277nm. The range of independent variables used for the optimization were MeOH: 60-70%, pH: 2-2.5 and flow rate: 0.3-0.8ml/min. The influence of these independent variables on the output responses: capacity factor of the first peak (k1), resolution (Rs) and separation (α) of the second peak and retention time (tr3) were evaluated. Using this strategy, mathematical model was defined, and response surface were derived for the separation. The coefficients of determination R² were more than 0.9258 for all the models. The four responses were simultaneously optimized by using Derringer's desirability functions and MCDM approach. Optimum conditions chosen for assay were MeOH, 0.01mM KH₂PO₄ (pH 2.5±0.5) adjusted with diluted orthophosphoric acid solution (68.03:31.97v/v) and flow rate of 0.8 mL/min. Peak area ratio of the analyte and internal standard was used for the quantification of pharmaceutical formulation samples. Total chromatographic analysis time per sample was approximately 9.0 min with DST, MPB (IS) and NLT eluting with retention times of 2.7, 3.2, and 6.0 minutes respectively. The optimized assay condition was validated as per ICH guidelines and applied for the quantitative analysis Sprycel-DST tablet and Tasigna-NLT capsule.

Key Words: Multi Criteria Decision Making Approach, Derringer' desirability function, Tyrosine Kinase Inhibitors, RP- HPLC, Dasatinib and Nilotinib.

INTRODUCTION

The deregulation of protein kinase has been identified to play a key role in the molecular pathogenesis of human cancers, such as chronic myelogenous leukemia and also in solid tumors¹. In Chronic myeloid leukemia (CML) tyrosine kinase receptors are proteins playing an important role in the transduction of the signals involved in growth of cells². CML is a myeloproliferative disorder characterized by the presence of the Philadelphia chromosome, a consequence of a reciprocal translocation between the arms of chromosomes 9 and 22, producing a fusion oncogene referred to as BCR-ABL (Break point cluster region-Abelson proto oncogene)^{3,4}. This BCR-ABL kinase is responsible for the activation of signal transduction cascades and consequently for uncontrolled cell proliferation, apoptosis and adhesion⁵. The first generation drug used for the treatment of CML is imatinib. The failure of imatinib most likely arises from a combination of tumor and host related factors that contribute to pharmacokinetic variability and induction of resistance⁶. Dasatinib and Nilotinib has revolutionized the treatment of chronic myeloid leukemia and tumors. These drugs are second generation, approximately more potent than imatinib and it also inhibits a number of imatinib-resistant mutant proteins⁷.

Dasatinib (DST), chemically N-(2-chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-methyl-4-pyrimidinyl]amino]-5-thiazole carboxamide monohydrate as shown in (Figure 1). Dasatinib is a white to off-white powder.

The solubility of drug substance is slightly soluble in ethanol, methanol, and polyethylene glycol and very slightly soluble in acetone, acetonitrile and practically insoluble in corn oil. Is a potent oral inhibitor of multiple oncogenic kinases⁸.

Nilotinib (NLT), chemically 4-methyl-N-[3-(4-methyl-1H-imidazol-1-yl)-5-(trifluoromethyl) phenyl]-3-[[4-(3-pyridinyl) amino] benzamide, monohydrate as shown in (Figure 2). Nilotinib is a white to slightly yellowish to greenish yellow powder. Nilotinib is soluble in organic solvents such as DMSO and dimethyl formamide (DMF), sparingly soluble in aqueous solutions. To enhance aqueous solubility, dilute the organic solvent solution into aqueous buffers and isotonic saline. Nilotinib is second generation tyrosine kinase inhibitor that is reported to have been used in targeted therapy for cancer⁹⁻¹⁰. Literature review reveals that some analytical methods have been reported for Dasatinib¹¹⁻¹³ and Nilotinib¹⁴⁻¹⁶ individually as stability indicating and in biological fluids or in combination with other drugs in pharmaceutical dosage forms. Recently HPLC, HPTLC and HPLC-MS¹⁷⁻²⁰ has been reported for the simultaneous determination of Dasatinib and Nilotinib in pharmaceutical dosage forms and biological fluids which are either tedious or expensive methods.

To the best of our knowledge, currently there is no HPLC method employing optimization techniques using MCDM approach have been reported for the simultaneous estimation of DST and NLT. Therefore, the simultaneous determination of these analytes

becomes encouraging and important. Developing and optimizing an isocratic HPLC^{21,22} method is a complex procedure that requires simultaneous estimation of several factors, viz., the type and composition of the organic phase, flow rate, pH, type of stationary phase, column temperature, etc. For decades HPLC separation were based on a trial and error methodology, but employing a time-consuming trial-and-error approach resulting only in an apparent optimum and information concerning the sensitivity of the factors on the analytes separation and interaction between factors is not available. To achieve this objective any one of the Chemometrics methods which includes the overlapping resolution maps²³ MCDM, factorial design²⁴ and response surface methodology²⁵⁻³² can be applied. The best experimental design approach for the purpose of modeling and optimization are the response surface design.

MATERIALS AND METHODS

Apparatus

Chromatographic measurements were made on a UFLC Shimadzu (Tokyo, Japan) model which consisted of a LC-20AD solvent delivery module, SPD-m20A prominence diode array detector, a Rheodyne injector (model 7125, USA) valve fitted with a 20 μ l loop. The system was controlled through a system controller (SCL-10A) and a personal computer using a Shimadzu chromatographic software (LC Solution, Release 1-11SP1) installed on it. The mobile phase was degassed using Branson sonicator (Branson Ultrasonic Corporation, USA). Absorbance spectra were recorded using an UV-double beam spectrophotometer (Systronics 2202 Model UV-1601PC, Japan) employing quartz cell of 1.00 cm of path length.

Software

Experimental design, data analysis and desirability function calculations were performed by using trial of version 10 of Design-Expert® Software. The calculations for the analysis were performed by use of Micro soft Excel 2007 software (Microsoft, USA).

Chemicals and reagents

Working standards of Dasatinib, nilotinib were donated by Hetero pharma, and Methyl paraben as shown in (Figure 3) was donated by sunglow pharma, Puducherry, India. Methanol [MeOH] of HPLC grade and potassium di hydrogen orthophosphate and orthophosphoric acid was of analytical- reagent grade supplied by M/S SD fine Chemicals, Mumbai, India. The HPLC grade water was prepared by using Milli-Q Academic, Millipore, and Bangalore, India. The pharmaceuticals Sprycel-DST tablet 100mg and Tasigna-NLT capsules 200mg were donated from Hetero pharma, Hyderabad, India.

Standard solutions

Stock standard solutions of DST, NLT and MPB (IS) (1mg/ml) were prepared in mobile phase. The prepared stock solutions were stored at 4°C protected from light. Working standard solutions were freshly obtained by diluting the stock solutions with mobile

phase during analysis day. Calibration curves reporting peak area ratios of DST, NLT to that of the MPB (IS) versus drug concentrations were established in the range of 10-50 μ g/ml in the presence of Methyl Paraben 30 μ g/mL as internal standard. Standard solution prepared for the optimization procedure constituted DST, NLT and MPB (IS) at 10.0, 10.0 and 30.0 μ g/mL respectively.

Sample preparation

Weigh and powder 10 tablets of DST and transfer tablet powder equivalent to 100mg of Dasatinib into a 200mL of volumetric flask, add 120mL of mobile phase and sonicate for not less than 30minutes with occasional shaking. Make up the volume to 200mL with mobile phase and mix. Centrifuge a portion of the above solution at 3000 rpm for 10 minutes and Filter the solution through 0.2 μ m membrane filter (Pall Life Science, India). Transfer 5.0mL of above solution in to a 25mL volumetric flask, dilute to volume with mobile phase and mix.

Weigh and empty 10 capsules of NLT and transfer capsule powder equivalent to about 100mg of Nilotinib into a 100mL of volumetric flask, add 60mL of mobile phase and sonicate for not less than 30 minutes with occasional shaking. Make up the volume to 100mL with mobile phase and mix. Centrifuge a portion of the above solution at 3000 rpm for 10 minutes and filter the solution through 0.2 μ m membrane filter (Pall Life Science, India).and discard first few mL of the filtrate. Further transfer 5.0ml of the above solution into a 100ml volumetric flask, dilute to volume with mobile phase and mix.

Chromatographic procedure

Chromatographic separation was carried out on a Phenomenex® C18 Enable column (150mm \times i.d., 5 μ m) connected with a Phenomenex® C18 guard cad ridge (4mm \times 3mm i.d., 5 μ m). The mobile phase consisted of MeOH: 0.01mM Potassium Dihydrogen Orthophosphate buffer (pH 2.5), adjusted with freshly prepared 10% orthophosphoric acid. Wavelength of 277 nm was selected for detection. An injection volume of the sample was 20 μ l. The HPLC system was used in an air-conditioned laboratory at atmosphere (20 \pm 2°C).

Validation

Validation studies were conducted using the optimized assay conditions based on the principles of validation described in the ICH guidelines "Text on validation of Analytical Procedures"³³ and "Q2B, Validation of Analytical Procedure: Methodology"³⁴. Key analytical parameters, including, accuracy, precision, linearity, detection limit, quantitation limit was evaluated. The calibration curves were tested using one-way ANOVA at 5% significance level. Calibration curves were constructed in a low region of 10-50% of the target analyte concentration for the limit of detection and quantification. Also, robustness of the proposed method was assessed with respect to small alterations in the MeOH concentration, pH, and buffer concentration

Table 1: Experimental design and results of a rotatable central composite Design ^a

Design points	Factor levels			Responses			
	A % v/v	B pH	C mL min ⁻¹	K ₁	Rs _{2,3}	α _(1,2)	tR ₄
1	65.00	1.83	0.55	0.678	1.4975	1.398	6.8
2	70.00	2.50	0.80	0.464	2.1605	1.5925	5.8
3	70.00	2.00	0.80	0.689	0.162	1.1235	5.8
4	65.00	2.25	0.55	0.731	2.0315	1.3155	9.5
5	70.00	2.50	0.30	0.469	2.61	1.599	15.7
6	65.00	2.25	0.55	0.731	2.0315	1.355	9.5
7	65.00	2.25	0.13	0.749	2.324	1.317	42
8	60.00	2.00	0.80	1.5855	0.000	0.000	7.3
9	65.00	2.25	0.55	0.731	2.0315	1.355	9.5
10	65.00	2.25	0.97	0.743	1.553	1.309	5.6
11	60.00	2.50	0.30	1.114	0.000	0.000	31.25
12	65.00	2.25	0.55	0.731	2.0315	1.355	9.5
13	60.00	2.00	0.30	1.609	0.000	0.000	24.8
14	73.41	2.25	0.55	0.362	0.000	0.000	6.1
15	65.00	2.25	0.55	0.731	2.0315	1.355	9.5
16	65.00	2.67	0.55	0.772	0.000	0.000	15.44
17	60.00	2.50	0.80	1.3105	0.000	0.000	12.5
18	56.59	2.25	0.55	1.564	1.725	2.536	19.2
19	65.00	2.25	0.55	0.731	2.0315	1.355	9.5
20	70.00	2.00	0.30	0.4695	2.61	1.599	15.5

^a Randomized

Table 2: Response models^a and statistical parameters obtained from ANOVA for CCD

Responses	Regression model	Adjusted R ²	Model P value	%C.V	Adequate precision
K ₁	+0.73-0.40* A-0.063* B+0.026* C+0.066* AB+2.688E-003* AC+1.937E-003* BC+0.11* A ² +0.030*B ² +0.037 * C ²	0.8590	<0.0002	16.79	13.51
Rs _(1,2)	+1.98-0.51* A-0.45* B-0.32* C+0.27* AB-0.38* AC+0.27* BC-0.45* A ² -0.49* B ² +0.27* ABC+0.72* A ² B+1.44* AB ²	0.9649	<0.0001	14.60	18.70
α _(1,2)	+1.36-0.75* A-0.42* B-2.378E-003* C+0.20* AB-0.20* AC+0.20* BC-0.13* A ² -0.33* B ² -0.11* C ² +0.20 * ABC+0.61* A ² B-0.20* A ² C+1.35* AB ² +0.000 (AC ² +*B ² C+ BC ² + A ³ + B ³ +* C ³)	0.7978	<0.0138	35.99	8.665
tR ₃	+9.77-4.03* A+1.93* B-8.57* C-1.43* AB+2.08* AC+0.79* A ² +4.73*C ²	0.9441	<0.0001	16.60	23.42

^a only significant coefficients with P < 0.05 are included. Factors are in coded levels.

Table 3: Criteria for the optimization of the individual responses for the analysis of quality control samples (Criteria I)

Responses	Lower limit	Upper limit	Criteria I		
			Goal	Importance	weights
Bn ⁷ K ₁	0.362	1.609	In range	3	1
Rs _(1,2)	0.000	1.751	Maximize	4	1
α _(1,2)	0.000	1.287	Maximize	3	1
tR ₃	5.6	42	Target=6	5	5

Table 4: Comparison of observed and predictive values of different objective functions under optimal conditions

Optimum conditions	MeOH(%)	pH	Flow (ml/min)	K ₁	Rs _(1,2)	α _(1,2)	tR ₃	
For Formulation	Desirability Value (D) = 0.808							
	68.03	2.5	0.8					
	Experimental value				0.56	1.81	1.39	5.84
	Predicted value				0.59	1.75	1.28	6.00
	Average % error				5.08	3.42	8.59	2.67

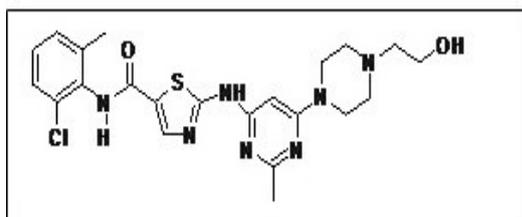


Figure 1: Dasatinib

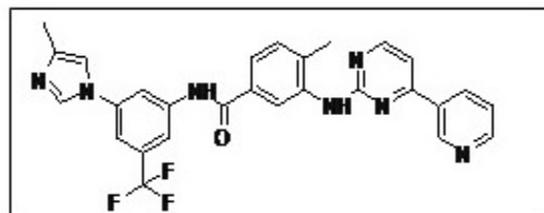


Figure 2: Nilotinib

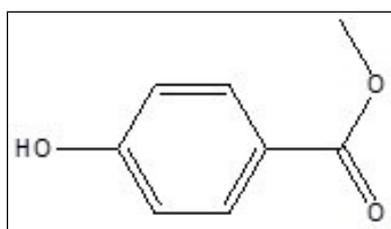


Figure 3: Methyl paraben

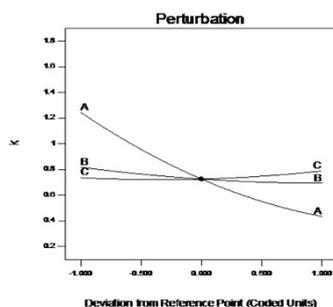


Figure 4a

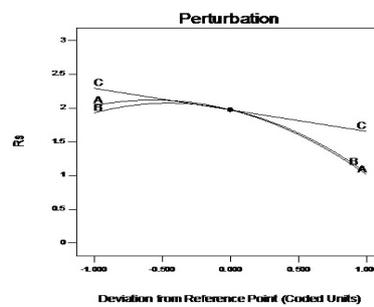


Figure 4b

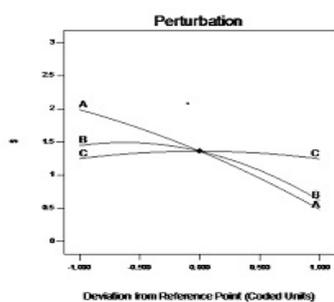


Figure 4c

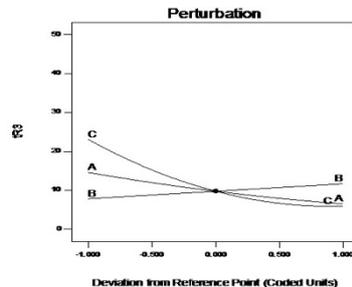


Figure 4d

Figure 4: Perturbation plots showing the effect of each independent variables on (a) k_1 , (b) $Rs_{(1,2)}$ (c) $\alpha_{(1,2)}$, (d) tR_3 Where A is the MeOH concentration, B the pH buffer, C the flow rate

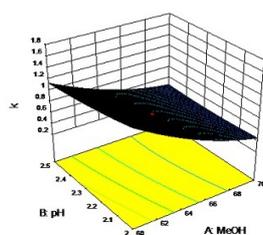


Figure 5a

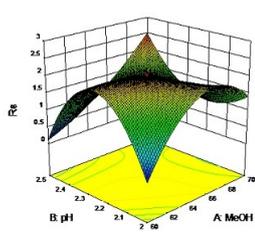


Figure 5b

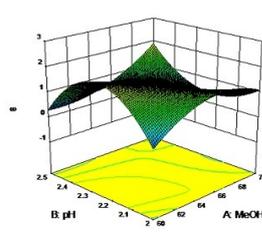


Figure 5c

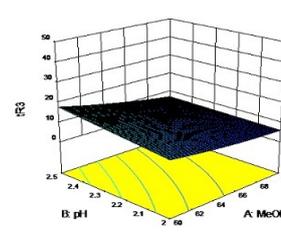


Figure 5d

Figure 5: Response surfaces related to MeOH (A) pH of buffer (B) Flow rate of mobile phase (C): (a) capacity factor first peak (k_1), (b) resolution of the critical pair $Rs_{(1,2)}$ (c) separation of $\alpha_{(1,2)}$ (d) retention time of the last peak (tR_3)

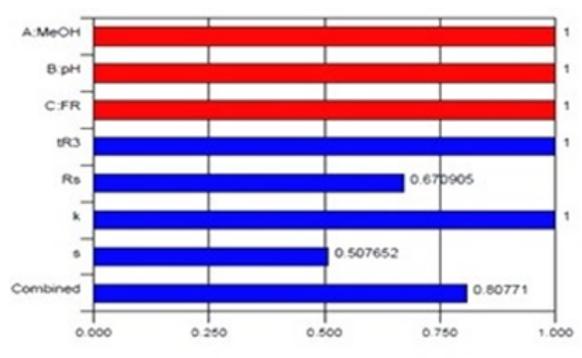


Figure 6: Graphical representation of the overall desirability function D (D=0.808) where MeOH Conc.(A) of 68.03, pH of buffer (b) 2.5 and Flow rate (c) 0.8mL/min and individual desirability of the four responses and three factors.

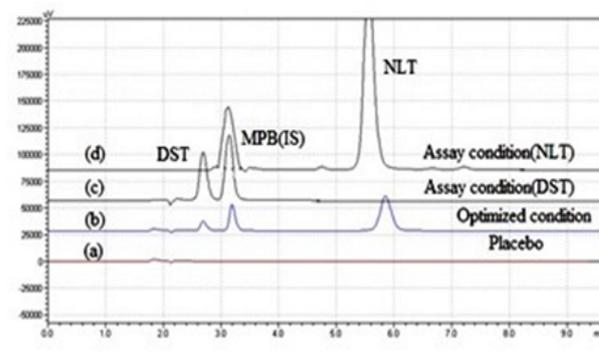


Figure 7: Chromatograms corresponding to (A) a Placebo solution; (B) Synthetic mixture of DST 10 µg/ml, IS (30 µg/ml), NLT (10 µg/ml); (C) a Real sample of Sprycel-DST tablet (40 µg/ml); (D) a Real sample of Tasigna-NLT tablet (50 µg/ml)

RESULTS AND DISCUSSION

Optimization design and analysis

Before starting an optimization procedure, it is important to investigate the curvature term using Factorial design with center points. ANOVA generated for 2^k Factorial design shows that curvature is significant for all the responses (k_1 , $R_{S(1,2)}$ and $\alpha_{(1,2)}$, tR_3) since p-value is less than 0.05. This implies that a quadratic model and cubic models should be considered to model the separation process. For resolution and separation models we selected cubic and for retention time, capacity factor we selected quadratic models. In order to obtain second order predictive model, central composite design (CCD) is employed, which is a design type under RSM. CCD is chosen due to its flexibility and can be applied to optimize an HPLC separation gaining better understanding of factors main and interaction effects. The selection of key factors examined for optimization was based on preliminary experiments and prior knowledge from literature. The factors selected for optimization process were MeOH concentration (A), pH of buffer (B) and flow rate (C). The capacity factor for first eluted peak (k_1), the resolution and separation of second peak ($R_{S(1,2)}$, α_2), the retention time of last peak (tR_3), was selected as responses. In the preliminary study, resolution between two peaks ($R_{S1,2}$), were found to be close to 0 and were merging, hence these two peaks were considered as critical peaks and we included resolution and separation of the second peak as responses for the global optimization. Methyl paraben was used as internal standard since it presented acceptable resolution and retention time with all the analytes.

All experiments were conducted in randomized order to minimize the effects of uncontrolled variables that may introduce a bias on the measurements. Replicates (n=6) of the central points were performed to estimate the experimental error. (Table 1), summarizes the conducted experiments and responses. The quadratic and cubic mathematical model for the independent factors is given in Eq. (1) & (2)

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad \text{Eq. (1)}$$

$$Y = \beta_0 x^3 + \beta_1 x^2 + \beta_2 x + \beta_3 \quad \text{Eq. (2)}$$

Where Y is the response to the modeled, β is the regression coefficient and X_1, X_2 and X_3 represents factors A, B and C,

respectively. Statistical parameters obtained from ANOVA for the reduced models are given in (Table 2). The insignificant terms ($P > 0.05$) were eliminated from the model through backward elimination process to obtain a simple and realistic model. Since R^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modeling adjusted R^2 which takes the number of regressor variables into account is usually selected.

In the present study, the adjusted R^2 were well within the acceptable limits of $R^2 \geq 0.80$ which revealed that the experimental data shows a good fit with the second-order polynomial equations. For all the reduced models, P value of < 0.05 is obtained, implying these models are significant. The adequate precision value is a measure of the signal (response) to noise (deviation) ratio". A ratio greater than 4 is desirable. In this study, the ratio was found to be in the range of 8.642 – 23.42, which indicates an adequate signal and therefore the model is significant for the separation process. The coefficient of variation (C.V.) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if it is less than 10%. The C.V. for all models was found to be less than 10%. The C.V. for all the models was found to be less than 10% except for $\alpha_{(1,2)}$ (35.99). Hence, the diagnostic plots, (a) normal probability plots³⁵ of residuals and (b) plot of residuals versus predicted values³⁶ were analyzed for response $\alpha_{(1,2)}$. Since the assumptions of normality and constant variance of residuals were found to be satisfied, the fitted model for the $\alpha_{(1,2)}$ was accepted.

As can be seen in (Table 2), the interaction term with the largest absolute coefficient among the fitted models is $A C (+2.08)$ of tR_3 model. The positive interaction between A and C is statistically significant (<0.0001) for tR_3 . The study reveals that changing the fraction of MeOH from low to high results in a rapid decline in the retention time of DST and NLT both at the low and high level of Ph. Further at low level of factor A, an increase in the Ph results in a marginal decrease in the retention time. Therefore, when the MeOH concentration has to be at its highest level to shorten the run time. Especially this interaction is synergistic, as it leads to a decrease in run time.

In (Figure 4) perturbation plots are presented for predicted models in order to gain an effect of an independent factor on a specific response with all other factor held constant at a reference point. A steepest slope or curvature indicates sensitiveness of the response to a specific factor. Figure 4d. Shows that Flow rate (factor C) and MeOH (factor A) had the most important effect on

a retention time tR_3 followed by pH (factor B). In Figure 4 b& c. The factors (pH, MeOH concentration & flow rate) had significant effect on $R_{s(1,2)}$ and $\alpha_{(1,2)}$ and only one factor A had significant effect on k_1 . In Figure 4a and (b), k_1 and $R_{s1,2}$ values increased as the level of MeOH concentration (factor A) decreased and $R_{s(1,2)}$ values increased at the level of buffer pH (factor B) and Flow rate (factor C) are at mid-point.

Response surfaces plots for k_1 , $R_{s1,2}$ and $\alpha_{1,2}$ and tR_3 are illustrated in Figure 5. (% Methanol concentration is plotted against the pH, Flow rate held at constant at the center value). Analysis of perturbation plots and response plots of optimization models revealed that factor A and B had the significant effect on a separation of the analytes, whereas the factor C i.e. the Flow rate, is of little significance.

Global optimization

In the present study the identified criteria for the optimization were: resolution between two critical peaks, capacity factor, separation and retention time of the last peak. Derringers desirability function was used to optimize three responses with different targets³⁷. The Derringers desirability function, D , is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions Eq. (3). The expression that defines the Derringers desirability function is:

$$\text{Eq. (3)} \quad D = \left[d_1^{p1} \times d_2^{p2} \times d_3^{p3} \times \dots \times d_n^{pn} \right]^{1/n}$$

Where p_i is the weight of the response, n the number of responses and d_i is the individual desirability function of each response. Desirability function (D) can take values from 0 to 1. Weights can range from 0.1 to 10. Weights lower than 1 gives less importance to the goal, whereas weights greater than 1 gives more importance to the goal. In the present study, p_i values were set 1 for K' , $R_{s(1,2)}$ and $\alpha_{(1,2)}$ responses and p_i for tR_3 was set to 5. A value of D close to 1 indicates that the combination of the different criteria is matched in a global optimum. In (Table 3). Criteria I have been proposed for selecting an optimum experimental condition for analyzing routine quality control samples. As can be seen under criteria I, the responses tR_3 was targeted to 6.0 min, in order to shorten the analysis time. On the other hand, $R_{s(1,2)}$ maximized to allow baseline separation DST and IS. In order to separate the first eluting peak (DST) from the solvent front, k_1 was in range. Importance can range from 1 to 5, which gives emphasis to a target value. The Importance for retention time is 5 to reduce the time of analysis. Following the conditions and restrictions above, the optimization procedure was carried out. The Graphical representation as shown in (Figure.6) of the overall desirability function D ($D=0.808$) where MeOH Conc.(A) of 68.03, pH of buffer (B) 2.5 and Flow rate (C) 0.8mL/min and individual desirability of the four responses and three factors. The predicted response values corresponding to the latter value of D were: $k_1=0.596$, $R_{s1,2}=1.751$, $\alpha_{(1,2)} = 1.287$ and $tR_2= 6.0$ min. The prediction efficiency of the model was confirmed by performing the experiment under the optimal condition and the corresponding chromatograms is shown in (Figure. 7).

In order to investigate the predictability of the proposed model, the agreement between experimental and predicted responses for the predicted optimums I are shown in (Table 4). The Percentage of prediction error was calculated by Eq. (4). The average error for $k'=5.08$ $R_{s(1,2)}=3.42$ $\alpha_{(1,2)}=8.59$ and $tR_3= 2.67$ were respectively, indicating good correlation between the experimental and predicted responses.

$$\text{Predicted Error} = \frac{\text{Experimental} - \text{Predicted}}{\text{Predicted}} * 100 \quad \text{Eq. (4)}$$

Assay method validation

The last step of the study was to check method validation for specificity, linearity, intra/inter-day precision, and robustness. The optimized HPLC method was specific in relation to the placebo used in the study. All placebo chromatograms showed no interference peaks (Figure 7a). An excellent linearity was established at five level in the range of 10-50 $\mu\text{g/ml}$ for DST&NLT and 30 $\mu\text{g/ml}$ of IS with R^2 of more than 0.998 for all the analytes. The slope and intercept of the calibration curve were. 0.009 and + 0.0576 for DST and 0.053 and 0.018 NLT respectively. Since the correlation coefficients are not good indicators of linearity performance of an analytical procedure a one-way ANOVA was performed. For all the analytes, the calculated F- Value (F_{calc}) was found to be less than the theoretical F-value (F_{crit}) at 5% significance level, indicating that there was no significance difference between replicate determinations for each concentration level. The LODs were 56.1, 5.22 ng/mL and LOQs were 170, 15.84ng/mL for DST and NLT respectively. Accuracy ($n=9$), assessed by spike recovery, were found to be 99.7, 97.7, for DST& NLT respectively, with were within acceptable ranges of $100 \pm 2\%$. The intra and inter-assay precision ($n=6$) was confirmed since, the %CV were well within the target criterion of ≤ 2 and ≤ 3 , respectively. Robustness study reveals that small changes did not alter the retention times, retention factor, and resolution and therefore it would be concluded that the method conditions are robust.

Application of the method

As a last step, commercial product of DST-Sprycel tablet 100 mg and commercial capsules of NLT- Tasigna 200 mg were assayed by the proposed HPLC method. Representative chromatograms are presented in figure7. The results achieved when analyzing DST-Sprycel tablets was 99.98 (0.42) mg of DST and when analyzing NLT-Tasigna capsules was 199.88 (0.62) mg of NLT. Good agreement was found between the assay results and the label claim of the product. The %C.V. for the tablet and capsules were < 2 , indicating the precision of the analytical methodology.

CONCLUSION

An efficient isocratic reversed-phase high-performance liquid chromatography method was developed, optimized and validated for the simultaneous estimation of Second generation TKI's namely DST and NLT in bulk and pharmaceutical formulations using Chemometrics Multi Criteria Decision Making Approach. This method reduces overall assay development time and provides essential information regarding the sensitivity of various chromatographic factors and their interaction effects on the attributes of separation. Time of analysis, resolution, and quality of the peaks were simultaneously optimized by applying useful tools of Chemometrics: central composite design and Derringers desirability function. The validation study supported the selection of the assay conditions by confirming that the assay was specific, accurate, and linear, precise, and robust. Therefore, this HPLC method can be used as a routine quality control analysis of second generation tyrosine kinase inhibitors.

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REFERENCES

- Pursche S, Ottmann O G, Ehninger G, Schleyer E. High-performance liquid chromatography method with ultraviolet detection for the quantification of the BCR-ABL inhibitor nilotinib (AMN107) in plasma, urine, culture medium and cell preparations. *Journal of Chromatography B* 2007; 208–216.
- Iris Garrido-Cano, Aurelio Garcia-Garci, Juan Peris-Vicente, Enrique Ochoa-Aranda, Josep Esteve-Romero. A method to quantify several tyrosine kinase inhibitors in plasma by micellar liquid chromatography and validation according to the European Medicines Agency guidelines. *Talanta* 2015; 144: 1287–1295.
- Ross DM, Hughes TP. Current and emerging tests for the laboratory monitoring of chronic myeloid leukemia and related disorders. *Pathology* 2008; 40:231-246.
- Masatomo Miura I, Naoto Takahashi and Ken-ichi Sawada. Quantitative Determination of Imatinib in Human Plasma with High-Performance Liquid Chromatography and Ultraviolet Detection. *Journal of Chromatographic Science* 2011; 49:
- Eva Kralj, Jurij Trontelja, Tadej Paji, Albin Kristl. Simultaneous measurement of imatinib, nilotinib and dasatinib in dried bloodspot by ultra-high-performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography B* 2012; 903:150– 156.
- Apperley JF. Mechanisms of resistance to imatinib in chronic myeloid leukaemia. *The lancet oncology* 2007; 8:1018-1029.
- Irina Andriamanana, Ines Gana, Benedicte Duret, Anne Hulin. Simultaneous analysis of anticancer agents bortezomib, imatinib, nilotinib, dasatinib, erlotinib, lapatinib, sorafenib, sunitinib and vandetanib in human plasma using LC/MS/MS. *Journal of Chromatography B* 2013; 926:83– 91
- Mhaske D V, Dhaneshwar S R. Stability Indicating HPTLC and LC Determination of Dasatinib in Pharmaceutical Dosage Form. *Chromatographia* 2007; 6695–102.
- Golemovic M, Verstovsek S, Giles F. *Clinical cancer research* 2005; 11:4941-4947.
- Weisberg E, Manley P, Mestan J. *British Journal of Cancer* 2006; 94:1765-1769.
- Bandi Ramachandra and N V S Naidu. Validation of rp-hplc method for estimation of dasatinib in bulk and its pharmaceutical dosage forms. *International journal of pharmaceutical and biological sciences* 2014; 4:61-68.
- Sreedevi A, Lakshmana Rao. Development and validation of novel hplc method for the estimation of dasatinib in bulk and pharmaceutical dosage forms. *International journal of Research in pharmacy and Chemistry* 2013; 3:724-729.
- Rama Krishna E. Development and Validation of an Improved Liquid Chromatography Method for the Determination of Dasatinib in Pharmaceutical Dosage Form. *International Journal of Medicine and Nanotechnology* 2015; 2:207-211.
- Fouad M A, Elkady E F. Forced Degradation Study and Validated Stability-Indicating RP-LC Method for Determination of Nilotinib in Bulk and Capsules. *Acta Chromatographica* 2014; 26:637–647.
- Myneni Harika, Kumar G S. Development and Validation of method for the determination of Nilotinib by RP-HPLC in Bulk and pharmaceutical dosage forms. *International Research Journal of Pharmacy* 2012; 3:161-164.
- Ramu ivaturi, Manikya Sastry T, Satyaveni S. Development and Validation of a Stability indicating HPLC method for the Determination of Nilotinib Hydrochloride in Bulk and Pharmaceutical dosage form. *International Journal of Pharmacy and Pharmaceutical Sciences* 2016; 8:41-48.
- De Francia, Antonio D Avolio, Francesca De Martino, Elisa Pirro, Lorena Baietto, Marco Siccardi, Marco Simiele, Silvia Racca, Giuseppe Saglio, Francesco Di Carlo, Giovanni DiPerri. New HPLC–MS method for the simultaneous quantification of the antileukemia drugs imatinib, dasatinib, and nilotinib in human plasma. *Journal of Chromatography B* 2009; 877:1721–1726.
- Nielka P van Erp, Djoeko de Wit, Henk-Jan Guchelaar, Hans Gelderblom, Trees J Hessing, Jan den Hartigh. validated assay for the simultaneous quantification of six tyrosinekinase inhibitors and two active metabolites in human serum using liquid chromatography coupled with tandem mass spectrometry. *Journal of Chromatography B* 2013; 937:33– 43.
- Antonio D’Avolio, Marco Simiele, Silvia De Francia, Alessandra Ariaudo, Lorena Baietto, Jessica Cusato, Carmen Fava, Giuseppe Saglio, Francesco Di Carlo, Giovanni Di Perri. HPLC–MS method for the simultaneous quantification of the antileukemia drugs imatinib, dasatinib and nilotinib in human peripheral blood mononuclear cell (PBMC). *Journal of Pharmaceutical and Biomedical Analysis* 2012; 59:109– 116.
- Andrea Davies, Alison K Hayes, Katy Knight, Sarah J Watmough, Munir Pirmohamed, Richard E Clark. Simultaneous determination of nilotinib, imatinib and its main metabolite (CGP-74588) in human plasma by ultraviolet high-performance liquid chromatography. *Leukemia Research* 2010; 34:702–707.
- Sree janardhanan V, Manavalan R and Vlliappan K. HPLC method for the simultaneous determination of proto pump inhibitors with domperidone in human plasma employing response surface design. *International Journal of Pharmacy and Pharmaceutical Sciences* 2012; 4:309-317.
- Senthil kumar T, Solairaj P and Thangathirupathi A. Analytical method development and Validation of donepezil hydrochloride tablets by RP-HPLC. *International Journal of Pharmacy and Pharmaceutical Sciences* 2011; 3:62-65.
- Lewis JA, Snyder LR and Dolan JW. Initial experiments in high-performance liquid chromatographic method development II. Recommended approach and conditions for isocratic separation. *Journal of Chromatography A* 1996; 721:15-29.
- Valliappan K, Kannan K, Manavalan R, and Muralidharan C. Application of chemometrics in chromatography. *Indian Journal of Chemistry* 2002; 41:7.
- Myers RH and Montgomery D. *Response Surface Methodology*. John Wiley & Sons Inc., New York; 1995.
- Sivakumar T, Manavalan R and Valliappan K. Global optimization using Derringer's desirability function: enantioselective determination of ketoprofen in formulations and in biological matrices. *Acta Chromatographica* 2007; 19: 29-47.
- Sivakumar T, Manavalan R, Muralidharan C and Valliappan K. Multi-criteria decision-making approach and experimental design as chemometric tools to optimize HPLC separation of domperidone and pantoprazole. *Journal of Pharmaceutical and Biomedical Analysis* 2007; 43:1842–1848.
- Thaniachalam Sivakumar, Rajappan Manavalan, Chandrashekar Muralidharan and Kannappan Valliappan. An improved HPLC method with the aid of a chemometric protocol: simultaneous analysis of amlodipine and

- atorvastatin in pharmaceutical formulations. Journal of Separation Sciences 2007; 30:3143-3153.
29. Sivakumar Thanikachalam, Manavalan Rajappan and Valliappan Kannappan. Stability-Indicating HPLC Method for Simultaneous Determination of Pantoprazole and Domperidone from their Combination Drug Product. Journal of Chromatographia 2008; 67:41-47.
 30. Suresh R, Manavalan R and Valliappan K. Developing and Optimizing a Validated RP-HPLC method for the analysis of amlodipine and ezetimibe with atorvastatin in pharmaceutical dosage forms applying response surface methodology. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4:550-558.
 31. Ramalingam Suresh, Rajappan Manavalan and Kannappan Valliappan, HPLC method for the simultaneous determination of Levocetirizine, Ambroxol and Montelukast human Plasma employing response surface Methodology. International Journal of Drug Development & Research 2012; 4: 173-185.
 32. Suresh R, Manavalan R and Valliappan K. Chemometric Assisted RP-HPLC Method for the Simultaneous Determination of Levocetirizine, Ambroxol and Montelukast in pharmaceutical Formulation. International Journal of Pharmaceutical and Chemical Sciences 2012; 1:1205-1220.
 33. Proceedings of the International Conference on Harmonization (ICH), Q2B: Text on Validation of Analytical Procedures: Definitions and Terminology, US FDA Federal Register; 1995.
 34. Proceedings of the International Conference on Harmonization (ICH), Q2B: Text on Validation of Analytical Procedures: Methodology, US FDA Federal Register; 1997.
 35. Wai- Bun Lui B and Peng. Composition optimization of extruded starch using response surface methodology. Journal of Packaging Technology and Science 2004; 17:6.
 36. Barmpalexis P, Kanaze F I and Georgarakis E. Developing an optimizing a validated isocratic reversed phase HPLC separation of nimodipine and impurities in tablet using experimental design methodology. Journal of Pharmaceutical and Biomedical Analysis. 2009; 49:5.
 37. Derringer G and Suich R. Simultaneous optimization of several response variables. Journal of Quality Technology 1980; 12:214-219.

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