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Method Development and Validation for Simultaneous Estimation of Dasatinib and Erlotinib by Using RP-HPLC in Pharmaceutical Dosage Form

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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 Erlotinib-ERT) 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 ERT 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 Tarceva -ERT capsule.

KEYWORDS: *Multi Criteria Decision Making Approach, Derringer' desirability function, Tyrosine Kinase Inhibitors, RP- HPLC, Dasatinib and Erlotinib*

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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 tumors[1]. In Chronic myeloid leukemia (CML) tyrosine kinase receptors are proteins playing an important role in the transduction of the signals involved in growth of cells[2]. CML is amyeloproliferative 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 adhesion[5].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 resistance[6]. Dasatinib and Nilotinb 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 proteins[7].

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 kinases[8]. Erlotinib (ERT), chemically N-(3-ethynylphenyl)-6, 7-bis(2-methoxyethoxy) quinazolin-4-amine as shown in (Figure 2). Erlotinib is a white to slightly yellowish to greenish yellow powder. Erlotinib is soluble in organic solvents such as DMSO and dimethyl form amide (DMF), sparingly soluble in aqueous solutions. To enhance aqueous solubility, dilute the organic solvent solution into aqueous buffers and isotonic

saline. Erlotinib is second generation tyrosine kinase inhibitor that is reported to have been used in targeted therapy for cancer [9-10]. Literature review ravels that some analytical methods have been reported for Dasatinib [11-13] and Erlotinib [14-16] individually as stability indicating and in biological fluids or in combination with other drugs in pharmaceutical dosage forms. Recently HPLC, HPTLC and HPLC-MS [17-20] has been reported for the simultaneous determination of Dasatinib and Erlotinib 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 ERT. 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 maps [23] MCDM. factorial design [24] and response surface methodology[25-32] 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 20µl 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 (Systronices 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, Erlotinib 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 Tarceva- ERT capsules 200mg were donated from Hetero pharma, Hyderabad, India.

Standard solutions

Stock standard solutions of DST, ERT 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, ERT to that of the MPB (IS) versus drug concentrations were established in the range of 10-50µg/ ml in the presence of Methyl Paraben 30µg/mL as internal standard. Standard solution prepared for the optimization procedure constituted DST, ERT and MPB (IS) at 10.0, 10.0 and 30.0 µg/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 0.2µm 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 ERT and transfer capsule powder equivalent to about 100mg of Erlotinib 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 0.2µm 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× i.d., 5µm) connected with a Phenomenx® C18 guard cad ridge (4mm×3mm i.d., 5µm). 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 20µl. The HPLC system was used in an air-conditioned laboratory at atmosphere (20±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: Methadology"[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^2B+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* ^{2}+0.20 * ABC+0.61* A^2B-0.20* A^2C+1.35* AB^2+0.000 (AC^2 +*B^2C+ 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'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

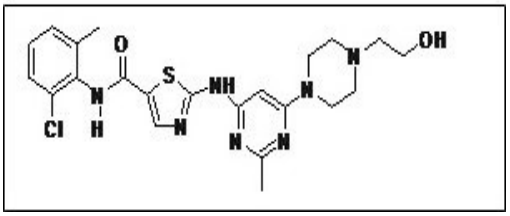

Figure 1: Dasatnib

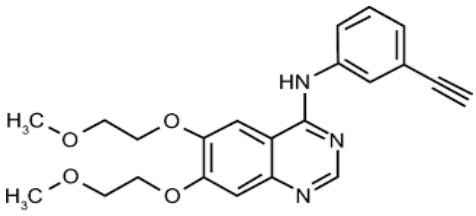

Figure 2: Erlotinib

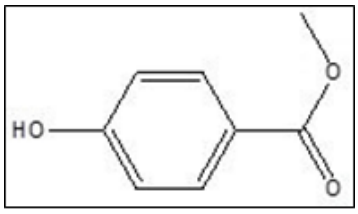

Figure 3: Methyl paraben

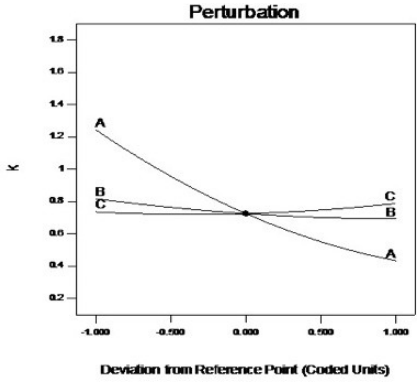

Figure 4a

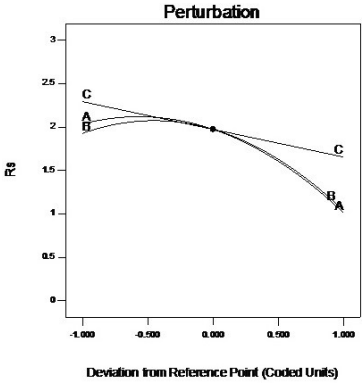

Figure 4b

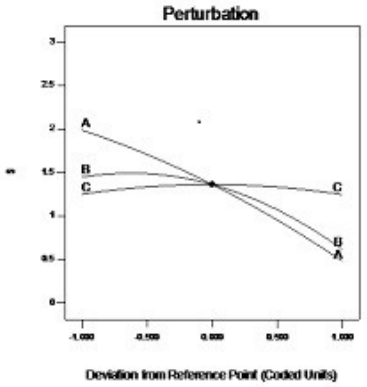

Figure 4c

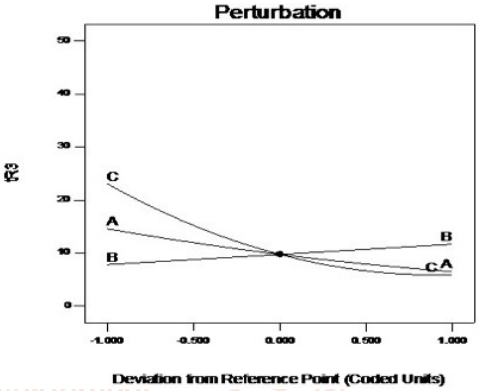

Figure 4d

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

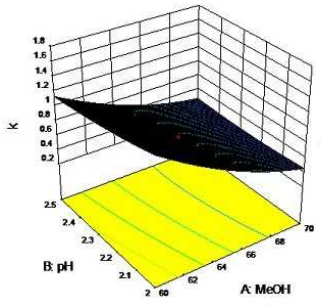

Figure 5a

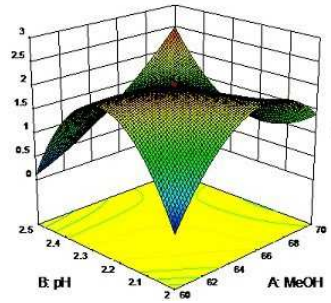

Figure 5b

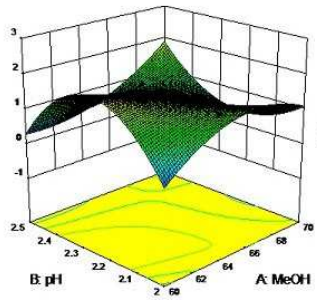

Figure 5c

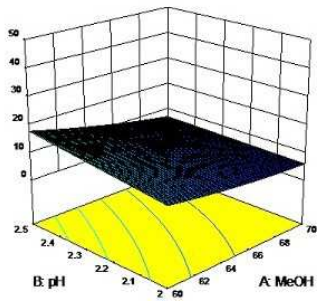

Figure 5d

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 α (1, 2) (d) retention time of the last peak (tR_3)

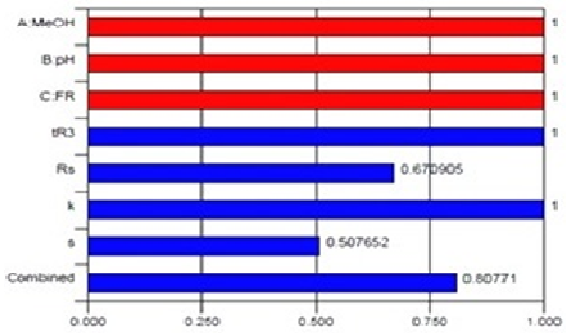

Figure 6: Graphical representation of the overall desirability function D (D=0.808) where MeOH Conc.(A) of 68.03, pH of buffer (b) 2.5 and Flow rate (c) 0.8mL/min and individual desirability of the four responses and three factors.

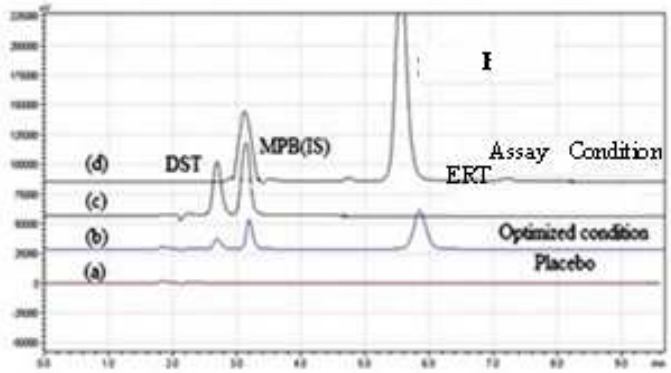

Figure 7: Chromatograms corresponding to (A) a Placebo solution; (B) Synthetic mixture of DST 10 µg/ml), IS (30 µg/ml), ERT (10 µg/ ml); (C) a Real sample of Sprycel-DST tablet (40 µg/ml); (D) a Real sample of Tarceva-ERT tablet (50 µg/ml)

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 α (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 ($R_{s\,(1,\,2),}$ α2), the retention time of last peak (tR3), was selected as responses. In the preliminary study, resolution between two peaks (Rs1, 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)

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Eq. (1)

$$Y = \beta 0x^3 + \beta 1x^2 + \beta 2x + \beta 3 \quad \textbf{Eq. (2)}$$

Where Y is the response to the modeled, β is the regression coefficient and $X_{1,\ X2}$ 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 lee 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 α $(_{1,\ 2})$ (35.99). Hence, the diagnostic plots, (a) normal probability plots[35] of residuals and (b) plot of residuals versus predicted values[36] were analyzed for response α $(_{1\,2})$. Since the assumptions of normality and constant variance of residuals were found to be satisfied, the fitted model for the α (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 ERT 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. Figure4d. 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 $Rs_{(1, 2)}$ and α (1, 2) and only one factor A had significant effect on k_1. In Figure 4a and (b), k_1 and $Rs_{1\,2}$ values increased as the level of MeOH concentration (factor A) decreased and $Rs_{(1,\ 2)}$, 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, $Rs_{1\,2}$ 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 targets[37]. 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:

Eq. (3) $D = [d^{p1} \times d^{p2} \times d^{p3} \times d^{pn}]^{1n/}$

Where *p*i is the weight of the response, *n* the number of responses and di 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 than1 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', Rs (1, 2) and α (1, 2) responses and pi for tR3 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, $Rs_{(1,2)}$ 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: k1=0.596, Rs=1.751, α (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 $Rs_{(1, 2)}$ =3.42 α (1, 2) =8.59 and tR3= 2.67 were respectively, indicating good correlation between the experimental and predicted responses.

Predicted Error= Experimental- Predicted/ Predicted *100 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 µg/ml for DST&ERT and30µ 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 ERT 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 ERT respectively. Accuracy (n=9), assessed by spike recovery, were found to be 99.7, 97.7, for DST& ERT respectively, with were within acceptable ranges of 100± 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 change s 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 ERT- Tarceva 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 ERT-Tarceva capsules was

199.88 (0.62) mg of ERT. 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 ERT 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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