Original Article

Design of Experiments Approach to Discriminatory Dissolution Method Development of Poorly Soluble Drug in Immediate Release Dosage Form

Sudheer Babu Kolla[1,2,*], Madhusudhan Rao Vallabhaneni[1], Srinivasa Babu Puttagunta[3], Maharshi Sudulagunta Venkata[2]

[1]Department of Chemistry, Vignan's Foundation for Science, Technology and Research University, Vadlamudi, Guntur, Andhra Pradesh, INDIA.
[2]Department of Analytical Research and Development, Formulations, GVK Bio Sciences Pvt. Ltd, JP Nagar, Bangalore, Karnataka, INDIA.
[3]Department of Pharmacy, Vignan College of Pharmacy, Vadlamudi, Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, INDIA.

ABSTRACT

Aim: The study involved design of experiment guided discriminatory dissolution method development for poorly soluble, ezetimibe tablets. **Objective:** In the current scope of study, ezetimibe tablets are selected as a suitable drug product candidate to evaluate the application of design of experiments in discriminatory dissolution method development for poorly soluble drug. Ezetimibe is practically insoluble in all the aqueous buffers. **Methodology:** 2-Level factorial design is selected as suitable model to build the experimental setup. Different factors like pH of dissolution media, sodium lauryl sulphate concentration, dissolution media volume and agitation speed (RPM) are selected for the study and dissolution % release at 5, 10, 15, 20, 45 min and % RSD of dissolution values at 20 min were selected as responses based on prior experience. The responses are evaluated for statistical significance and for adequacy of the built design with the help of different tools like ANOVA and diagnostic graphs. **Results:** It is observed that pH of dissolution media, surfactant concentration are having minimal positive effect on all of the responses. Whereas agitation speed and dissolution media volume were having significant positive effect on all responses, except for % RSD at 20 min and is inversely proportional to agitation speed. **Conclusion:** With the current scope of study design of experiments as an effective tool for discriminatory dissolution method development is employed to prove adequacy. Selected solutions from predictions were executed for experimental results and were compared against predictions to validate model.

Key words: DoE, Discriminatory dissolution method, Ezetimibe tablets, DoE aided dissolution method development.

INTRODUCTION

To predict the similarity of test product to that of reference product of a pharmaceutical generic tablet dosage form, a dissolution profile comparison is performed under identical conditions for the product before and after the change(s) is recommended. Dissolution profiles are considered similar by virtue of overall profile similarity and similarity at every dissolution sample time point.[1] Dissolution as a tool for evaluation of *in-vitro* performance and equivalence of the test product to reference product, is a widely used tool in pharmaceutical industry for product development.[2,3] To compare any two different formulations performance in terms of dissolution characteristics, it is essential to first develop a dissolution method.[4] Discriminatory dissolution method is very essential to compare any two formulations performance in terms of dissolution characteristics[5] and should be a combination of right

Submission Date: 08-09-2018;
Revision Date: 28-12-2018;
Accepted Date: 21-03-2019

DOI: 10.5530/ijper.53.3.76

Correspondence:
Mr. Sudheer Babu Kolla,
Department of Chemistry, Vignan's Foundation for Science, Technology and Research University, Vadlamudi, Guntur, Andhra Pradesh- 522213, INDIA.
Phone: +91 9949894569
E-mail: sudheerchowdary1979@gmail.com

www.ijper.org

selection of parameters for dissolution testing like media volume, agitation speed (RPM), buffer pH and concentration of buffer (Dissolution media) along with the concentration of additives like surfactants in case of poorly soluble molecules.[6]

Discrimination power of the dissolution method is described as the ability of the dissolution test method to differentiate the performance of two different formulations with the release pattern.[7] The dissolution test method shall be able to identify the minor formulation differences and shall reflect the same in terms of changes in dissolution profile.[7,8] Different approaches are available in literature in order to achieve discriminatory dissolution method and most of them recommend the use of specified and limiting dissolution conditions like (1) Dissolution media volume, (2) Dissolution media pH, (3) Agitation rate of apparatus, (4) Concentration of surfactants if any.[7] The target is to achieve a slow release in the initial 15 min which gradual increase by 30 min of dissolution testing and achieves a complete release at about 45 to 60 min.[5] In order to achieve this criteria, the selected dissolution media and volume shall be sufficient enough to facility complete release and addition of surfactant is desired at certain concentration to facilitate solubility of poorly soluble molecules.[7] Increase in SLS concentration alone may cause sudden burst of drug release and discrimination cannot be achieved. In order to arrive at the optimum concentration of SLS and media volume with right combination of agitation speed (RPM) and pH of dissolution media, all the selected variables are to be optimized in combination to yield a discriminatory dissolution method. Bioequivalence (BE) studies are required to be carried out to prove the formulation equivalence of test product with that of the reference listed drug product and *in-vitro* dissolution profile matching will further enhance the success rate of BE studies.[9]

The current research work is aimed at studying the applications of DoE (Design of experiments) in developing a discriminatory dissolution method for poorly soluble Ezetimibe tablets. Not many studies were reported in literature on the methodologies of application of DoE in dissolution method development for poorly soluble molecules. Available literature references majorly focus on QbD implementation in dissolution method development,[10-12] DoE for extended release profile[13] and DoE in dissolution method optimization of modified release tablets.[14,15] For this study ezetimibe tablet which is in a class of lipid-lowering compound was selected, as the molecule is practically insoluble in water.[16] Ezetimibe is available as a tablet for oral administration containing 10 mg of ezetimibe and the following inactive ingredients: croscarmellose sodium NF, lactose monohydrate NF, magnesium stearate NF, microcrystalline cellulose NF, povidone USP and sodium lauryl sulfate NF.[16] Available literature methods for Ezetimibe tablets are for conventional QC release purpose and not enough discriminating for prediction of *in-vitro* equivalence of test and reference product.[17,18] Other literature reported dissolution methods were targeted for platform technology formulations but not for conventional IR formulations and without use of surfactants.[19] A '2-level factorial design' studies all the factors at high and low of the combinations and uses statistics to identify the critical factors and their influence on the responses being studies.[20] Thus with less number of runs/ experiments more information about influence on each factor can be predicted. Factorial design are simple to use and has been widely used in pharmaceutical analytical processes.[21-23]

MATERIALS AND METHODS

Chemicals and Reagents

Pure ezetimibe was supplied by Inogent Laboratories Private Limited (A GVK BIO Company-Hyderabad, INDIA). Ezetimibe tablets were supplied by GVK BIO, Formulations. Hydrochloric acid (HCl), Sodium dodecyl sulphate (SLS), sodium hydroxide pellets, glacial acetic acid, HPLC grade Acetonitrile (ACN) and methanol were procured from Merck Life Sciences Pvt. Ltd. (Mumbai, India). Buffer salts (Sodium acetate anhydrous, potassium chloride and monobasic potassium phosphate) and all other chemicals were of Emplura grade. Ultra-pure water was obtained from SG water purification system (Hyderabad, India).

Apparatus and Equipment

Dissolution apparatus were used for discriminatory dissolution method development studies. An auto sampler dissolution tester (TDT-14L, ELECTROLAB, Mumbai, India) and a manual dissolution apparatus (EDT-08LX, ELECTROLAB, Mumbai, India) with USP type –II (Paddle) accessories were used. pH was observed by using pH/Ion analyzer (LP139SA, Polmon, Bangalore, India). HPLC studies were carried out on Prominence-I LC2030 liquid chromatograph (Shimadzu, Kyoto, Japan), which was equipped with a Photo diode array detector and 1200 Series liquid chromatograph (Agilent Technologies Inc., Santa Carla, USA) equipped with a Photo diode array detector and UV detector. Zodiac C18 Column (150 mm x 4.6 mm, 5µ particle size, Zodiac Life Sciences, Hyderabad, India) was utilized in the study. Other equipment used were micro balance (ME 5, Sartorius, Switzerland), analytical balance (XB220A, Precisa Gravimetric AG, Dietikon, Switzerland) and Magnetic stirrer (Remi Equipment's private limited). Pipettes and remaining glassware were made of Borosil.

0.45µm Polyvinylidene Fluoride (PVDF) filters (Merck, Bangalore, India) were used for the filtration of sample solutions. Design-Expert 10 software was used during DoE studies so as to generate experimental designs and to analyze the obtained responses.

Procedure and Details of Test Product

Dissolution studies were performed with paddle apparatus by maintaining bath temperature at 37°C. All the dissolution media were prepared as directed in USP general chapters. Standard solution of ezetimibe is prepared by dissolving 20 mg of substance in 100 ml of methanol and further diluting the solution with respective dissolution media to attain a final concentration of 0.02 mg/ml of ezetimibe. Dissolution sample solutions at predefined intervals were withdrawn and filtered through 0.45 µ PVDF membrane filter. Test samples were obtained from GVK BIO, formulations, INDIA. All the solutions were analyzed by HPLC technique to estimate % amount of drug release on label claim at each interval. The experiments by HPLC were performed on Zodiac C_{18} Column (150 mm x 4.6 mm, 5µ), with a flow rate of 1.0 ml/min, UV detection wavelength of 233 nm. Mobile phase for HPLC analysis was prepared by mixing phosphate buffer solution (0.01 Molar) and acetonitrile in the ratio of 60:40 v/v.

Solubility Studies and Dissolution Studies with OFAT Approach

Ezetimibe API is reported to be practically insoluble in water.[16] The prescribed daily dose of ezetimibe tablets is 10 mg/day.[16] Solubility studies contribute a vital information on selection of dissolution media. Solubility studies were performed in different media across physiological pH range also by adding SLS at concentration of 0.2% and 0.5%. Preliminary dissolution studies were conducted in different dissolution media at pH 1.2, pH 4.5 and pH 6.8 in plain media also with 0.45% of SLS as additive to predict the behavior of ezetimibe tablets to form a basis of selection of dissolution media and method conditions. Considering maximum dissolution media volume of 900 ml, an approximate solubility of 0.01 mg/ ml is required to solubilize ezetimibe tablets. Further based on knowledge gained from OFAT experimental trials, to achieve a discriminatory and complete dissolution of ezetimibe tablets, different combination of pH of dissolution media, SLS concentration, agitation speed and media volume were experimented by DoE.

Application of DoE During Discriminatory Dissolution Method Development

Design of experiments considers multiple factors to be experimented in a single experiment and all the factors were varied in each of the set of experiments as per predetermined statistical modelling.[24] A simple 2-level factorial design was used to develop discriminatory dissolution method to achieve desired dissolution profile with high and low levels of each selected factor/ variable. In order to gain more information, the design was enabled with "center points" option to include mid values of each factor in the experimental design to check for lack of fit and curvature. Thus a total of 22 runs were arrived for experimentation including 6 center points.

Ezetimibe tablets were subjected to different combination of dissolution conditions by varying the method parameters such as pH of dissolution media (pH 1.2 to pH 4.5; considered as categorical variable), SLS concentration (0.2 % to 0.75%), dissolution media volume (250 ml to 900 ml) and agitation speed (RPM, 25 to 75). Paddle apparatus was selected for dissolution studies as the dosage form is tablet.[25,26] DoE was applied to dissolution method parameters and dissolution profile at 5, 10, 15, 20 and 45 min along with % RSD at 20 min was obtained on tablet dissolution.

Design Evaluation for Adequacy and Statistical Significance

To evaluate effectiveness of the experimental design, 2-level factorial design was assessed through a statistical measure of power, lack of fit and pure error. These 3 statistical parameters determine the adequacy of the design model created. Additional graphical evaluation was performed through fractional design space (FDS) graph. Statistical evaluation tools like Power lack of fit, pure error and VIF value are evaluated to ensure adequacy of the design.[27] Obtained results for lack of fit, pure error and power for all variables individually and in combination indicate that the experimental design is significant. As design was proven to be adequate, all the experimental runs were executed and results of each response were evaluated for statistical significance by ANOVA tool. ANOVA includes Model F–value, adjusted R-square, predicted R-square and adequate precision as statistical measures.[27]

Design Prediction and Validation

As the design model was proven to be statistically significant, further results were navigated for effects of variables on responses with the help of perturbation graph, contour graph and 3 D surface graphs to understand which variable is having significant effect on the responses. Next step of DoE involves prediction of solutions as per the desired outcome and validating the suggested solutions against experimental data. DoE design was aided with desired dissolution profile values at each time point as ranges of % dissolution and solu-

tions were predicted. Out of the suggested solutions, selected solutions were methodically evaluated with numerical optimization and overlay graph to understand the method operable design region (MODR) of experimental design.[28-30] Dissolution profile was established with suggested method conditions and the experimental results were compared against the DoE predicted values to validate the model.

RESULTS AND DISCUSSION

Solubility and Dissolution Studies as Preliminary Screening

Saturation solubility studies and dissolution profiles in different media at standard conditions were executed to establish a scientific basis of selection of variables and ranges for the DoE study. Solubility studies, at saturation level, of ezetimibe across physiological pH range in different pH buffer solutions with SLS as additive were conducted. SLS was considered as anionic surfactant additive to enhance solubility, as the molecule is practically insoluble in aqueous media. The observed solubility was giving sufficient saturation solubility at 0.5% of SLS concentration. The results of solubility studies are listed in Table 1.

As was evident from the solubility studies, ezetimibe is having very poor solubility in plain aqueous media across the physiological pH range. But it was observed that with increase in SLS concentration the solubility is increasing and at about 0.5% of SLS, sufficient solubility to achieve complete dissolution of dosage strength was attained. Dissolution studies were performed in different buffer solution across physiological pH range, with and without 0.45% SLS. The observed dissolution profile in plain buffer solutions was less than 5% and in 0.45% SLS in 0.05 molar pH 4.5 acetate buffer sudden burst release was observed in 10 min as well complete release was not observed. In other media with SLS, the release was slow and not complete. Results of dissolution studies with OFAT approach in different dissolution media are presented in Table 2.

Table 1: Solubility of Ezetimibe in Buffer Solutions Across Physiological pH Range (mg/ml).

SLS Conc. (% w/v)	Different pH buffers		
	A	B	C
0	0.001	0.003	0.002
0.1	0.002	0.001	0.003
0.2	0.003	0.004	0.006
0.5	0.007	0.012	0.017

SLS Conc: Sodium Lauryl Sulphate Concentration (% w/v); A, pH 1.2 hydrochloric acid buffer; B, pH 4.5 acetate buffer; C, pH 6.8 phosphate buffer.

Table 2: Dissolution Studies with OFAT Approach in Different Dissolution Media.

Ezetimibe Tablet 10mg, 500 ml dissolution media volume, 50 RPM agitation speed						
Time (min)	% Dissolution on label claim					
	A	B	C	D	E	F
5	1	1	2	0	49	0
10	1	1	1	3	71	31
15	1	1	2	4	78	41
20	2	1	1	15	83	59
30	2	1	1	19	86	64
45	1	2	4	42	89	75

A, pH 1.2 Hydrochloric acid; B, pH 4.5 Acetate buffer; C, pH 6.8 Phosphate buffer; D, 0.45% Sodium lauryl sulphate (SLS) in pH 1.2 Hydrochloric acid; E, 0.45% Sodium lauryl sulphate (SLS) in 0.05 M, pH 4.5 Acetate buffer; F, 0.45% Sodium lauryl sulphate (SLS) in pH 6.8 Phosphate buffer.

Design Adequacy and ANOVA

The design was evaluated for adequacy with statistical parameters. Observed degrees of freedom values for lack of fit and pure error were 3 and 4 respectively which indicate model adequacy. Power values were calculated for each variable and combination of different Variables which indicate the capability to detect signal to noise for each variable experiment. The observed power values were above 90% for all combinations of variable. All the statistical parameters were well within the desired limits signifying that the model built was statistically valid. Results of design adequacy evaluation are presented in Table 3. Additionally graphical evaluation tool of FDS graph was also evaluated. The recommended FDS score of at least 0.8 or 80% for exploration and optimization is desirable to yield better statistical modelling. Observed FDS score was 0.98 hence the design was adequate to create meaningful results. FDS graph of the executed DoE is shown in Figure 1.

Results of all the 22 experimental runs were fed to DoE software and were evaluated statistically for any anomalies. Experimental set along with ranges of variables and arrived responses for each run are presented in Table 4. Selected desired statistical model and evaluated the ANOVA parameters; Model F value, Lack of fit F-value, degree of freedom for pure error, *R*-squared values, adjusted *R*-square, predicted *R*-square and adequate precision. Model F-value for all the responses in found less than 5% for all responses which indicates that there are only about 5% or less chances that the model may fail due to noise. Lack of fit, F-Values were found to be more than the desired value of 3 for all the responses. The "Predicted R-squared" was not as close to the "Adjusted R-squared" and the difference was more than 0.2. "Adequate Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable.

Table 3: Design Evaluation Parameters for Adequacy.

Term	Std. Err	VIF	Power at 5 % alpha
A	0.21	1.00	97.1
B	0.25	1.00	91.2
C	0.25	1.00	91.2
D	0.25	1.00	91.2
AB	0.25	1.00	91.2
AC	0.25	1.00	91.2
AD	0.25	1.00	91.2
BC	0.25	1.00	91.2
BD	0.25	1.00	91.2
CD	0.25	1.00	91.2
ABC	0.25	1.00	91.2
ABD	0.25	1.00	91.2
ACD	0.25	1.00	91.2
BCD	0.25	1.00	91.2
ABCD	0.25	1.00	91.2
Lack of Fit	3	Pure Error	4

A, pH of dissolution media; B, Sodium lauryl sulphate concentration (%w/v); C, Dissolution media volume (ml); D, Agitation speed (RPM).

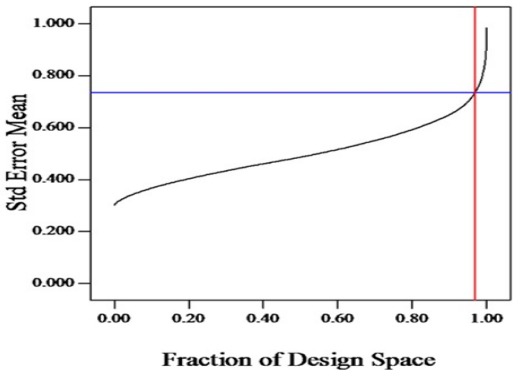


Figure 1: FDS Graph for Evaluation of Adequacy of the Model.

Observed ratio for all responses were above 4. ANOVA results evaluation table for statistical significance of model is presented in Table 5.

Evaluation of Responses (Perturbation Plots, Contour Plots, 3 D Plots)

All the responses were evaluated through different graphs to understand the effect and relation of variables on each response. Perturbation plot helps to compare the effects of all the factors at a particular point in the design space. Response 1 (% dissolution at 5 min) was responding positively to all variables; SLS concentration, dissolution media volume and agitation speed. Variable A, was not in the model as the same was considered as categorical and based on solubility studies pH 4.5 is selected as preferred for optimization of method. Figure 2 (a) depicts the relation of variables to response 1 and dissolution media volume was having more positive effect as increase in agitation speed will effect in tablet bursting effect thus aiding more release at initial time point. Figure 2 (b) shows the relation of variables to response 2 (% dissolution at 10 min) and all the variables were having positive effect. Agitation speed was having more positive effect on the release rate at 10 min which need to be optimized to have discriminatory release at initial time points. SLS concentration was having marginal positive effect on response 3 and 4 (% dissolution at 15 and 20 min) whereas dissolution media volume and agitation speed were having significant impact on release rate. Effect of variable on response 3 and 4 are depicted in Figure 2 (c), (d). Extent of release, i.e., % dissolution at 45 min was influenced to significant extent by dissolution media volume and agitation speed whereas SLS concentration was having nominal positive effect. Figure 2 (e) shows the relation of variables to response 5. Figure 2 (f) shows the relation of variables to response 6, where SLS concentration was having positive impact and all other variables were having negative impact. In order to achieve minimum possible % RSD at 20 min; target is to have lower value, it was desirable to have optimum values for dissolution media volume and agitation speed.

Further evaluation of responses with respect to variables was made with 3D Surface. 3 D surface plot allows to evaluate a combination selected 2 variables at time and evaluate the effect of variation on responses. Figure 3 shows the relation of selected variable (Dissolution media volume and SLS Concentration) on each response. It was observed that SLS concentration and dissolution media volume were having moderate positive effect on response 1 to 5 whereas agitation speed was having a high positive effect. As the objective was to achieve slow and steady dissolution profile, it was desirable to play with variable B and variable C.

Design Constraints for Solution Predictions and Optimization of Discriminatory Dissolution Method

In order to predict the solutions the software was given constraints which were target acceptance ranges or anticipated results for each of the response. For this purpose each of the factor was given with the targeted range to operate and then each of response was given with the constraint of desired result outcome. Table 6 illustrates the ranges of each of the variable and response. Response 1 was given a target of 10 to 35 % of dissolution release to prevent release dumping at initial time point. Response 2 was given a target of 20

Table 4: Experimental Setup by DoE for Discriminatory Dissolution Method Development.

Runs	Variables				Responses[a]					
	A	B	C	D	R1	R2	R3	R4	R5	R6[b]
1	4.5	0.75	250	75	35	53	59	62	70	8.8
2	4.5	0.75	900	25	51	64	69	69	74	23.3
3	1.2	0.475	575	50	44	63	70	74	80	8.5
4	1.2	0.2	900	75	46	58	67	73	87	3.6
5	1.2	0.75	900	25	22	34	37	39	42	32.5
6	1.2	0.75	900	75	72	89	93	96	99	1.8
7	4.5	0.2	900	75	37	55	73	79	91	3.9
8	4.5	0.475	575	50	55	71	79	79	84	8.9
9	1.2	0.2	250	25	1	2	3	4	6	20.4
10	4.5	0.75	900	75	63	93	98	100	102	1.4
11	1.2	0.475	575	50	57	72	80	86	92	3.3
12	4.5	0.2	250	25	1	2	4	5	7	12.8
13	4.5	0.475	575	50	41	64	72	76	81	9
14	1.2	0.75	250	75	28	40	45	51	64	12.2
15	1.2	0.75	250	25	2	3	4	5	9	31.6
16	4.5	0.475	575	50	55	71	79	79	84	8.9
17	1.2	0.2	250	75	15	23	29	32	42	28.3
18	1.2	0.2	900	25	9	14	17	19	23	6.7
19	4.5	0.2	900	25	19	31	43	48	59	18.1
20	4.5	0.2	250	75	20	34	41	45	57	17
21	1.2	0.475	575	50	57	72	80	86	92	3.3
22	4.5	0.75	250	25	2	3	4	5	8	0

A, pH of dissolution media; B, sodium lauryl sulphate concentration (%w/v); C, dissolution media volume (ml); D, agitation speed (RPM); R1, % dissolution @ 5 min; R2, % dissolution @ 10 min; R3, % dissolution @ 15 min; R4,% dissolution @ 20 min; R5, % dissolution @ 45 min; R6, % RSD for dissolution values at 20 min.

[a] % dissolution on label claim of dosage form.

[b] % RSD of replicate measurement of dissolution values.

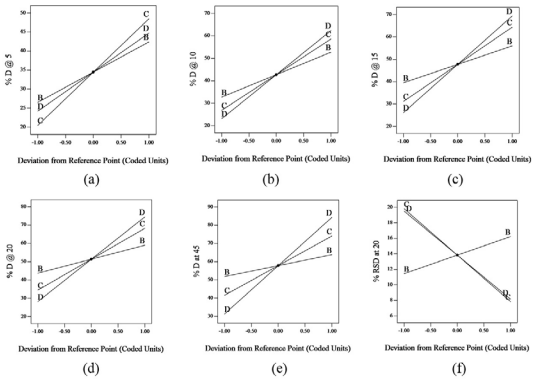


Figure 2: Perturbation Plots. (a) Perturbation plot for response-1: %D @ 5 min, (b) Perturbation plot for response-2: % D @ 10 min, (c) Perturbation plot for response-3: % D @ 15 min, (d) perturbation plot for response-4: % D @ 20 min, (e) Perturbation plot for response-5: % D @ 45 min, (f) Perturbation plot for response-6: % RSD @ 20 min.

Table 5: ANOVA Results Evaluation Table for Statistical Significance of Model.

Responses	Statistical parameters						
	A	B	C	D	E	F	G
R1	3.07	6.10	4	0.6975	0.4707	0.2114	6.812
R2	3.12	29.06	4	0.7392	0.5020	0.1655	6.486
R3	3.07	29.91	4	0.7363	0.4966	0.1635	6.315
R4	3.64	27.19	4	0.7320	0.5310	0.3079	6.820
R5	5.16	22.87	4	0.7605	0.6130	0.4802	7.871
R6	3.66	14.48	4	0.8009	0.5819	0.0800	7.187

R1, Response -1: % dissolution @ 5 min; R2, Response-2: % dissolution @ 10 min; R3, Response-3: % dissolution @ 15 min; R4, Response-4: % dissolution @ 20 min; R5, Response-5: % dissolution @ 45 min; R6, Response-6: % RSD for dissolution values at 20 min; A, model F-value; B, lack of fit F-value; C, pure error; D, R-squared; E, adjusted R- squared; F, predicted R-squared; G, adequate Precision.

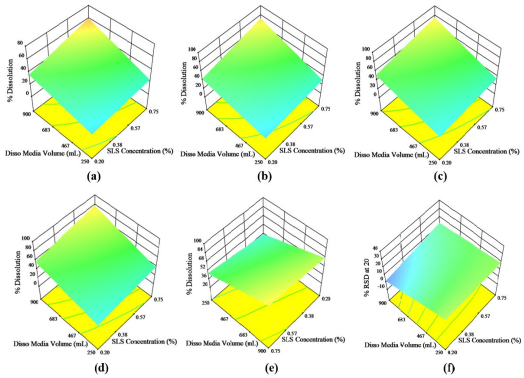


Figure 3: 3D Surface Plots. (a) 3D Surface plot for response-1: % dissolution @ 5 Min, (b) 3D surface plot for response-2: % dissolution @ 10 min, (c) 3D surface plot for response-3: % dissolution @ 15 min, (d) 3D surface plot for response-4: % dissolution @ 20 min, (e) 3D surface plot for response-5: % dissolution @ 45 min, (f) 3D surface plot for response-6: % RSD of dissolution values @ 20 min.

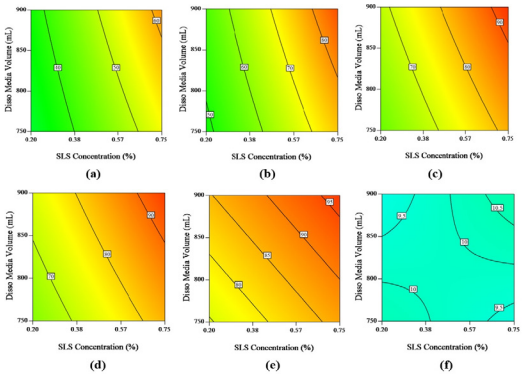


Figure 5: Contour Plots for 'Solution 13' (a) Contour plot for response-1: % dissolution @ 5 min, (b) Contour plot for response-2: % dissolution @ 10 min, (c) Contour plot for response-3: % dissolution @ 15 min, (d) Contour plot for response-4: % dissolution @ 20 min, (e) Contour plot for response-5: % dissolution @ 45 min, (f) Contour plot for response-6: % RSD of dissolution values @ 20 min.

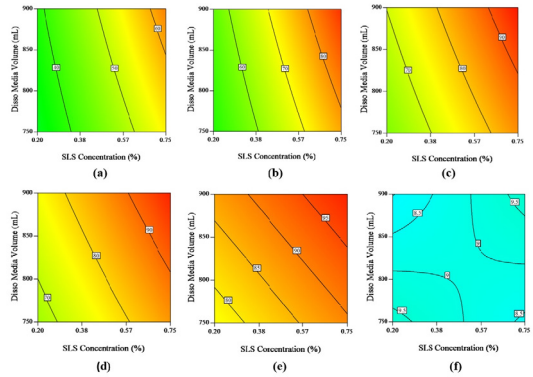


Figure 4: Contour Plots for 'Solution 1' (a) Contour plot for response-1: % dissolution @ 5 min, (b) Contour plot for response-2: % dissolution @ 10 min, (c) Contour plot for response-3: % dissolution @ 15 min, (d) Contour plot for response-4: % dissolution @ 20 min, (e) Contour plot for response-5: % dissolution @ 45 min, (f) Contour plot for response-6: % RSD of dissolution values @ 20 min.

to 70 to ensure that there is notable increase in release from 5 min to 10 min time point. Response 3 was given a target of 30 to 80 % of dissolution release to ensure that around 50% of the drug was released. Response 4 was given a target of 50 to 100 % of release to ensure that at least 50%. Response 5 was given a target of 75 % and above dissolution release to ensure that complete drug release was monitored. Response 6 was given a target less than 10% and objective to minimize.

Design Validation with Predicted and Selected Solutions

With the given target ranges and constraints for each of the responses, the software was able to suggest about 16 different solutions with a combination variable factors. Table 7 illustrates different solutions as proposed by software. As it was observed that pH of the dissolution media was having a very minimal effect and was negligible in terms of its influence on the observed results, pH 4.5 was selected as target pH as the same was relevant from physiological transit of the dosage form based on T_{max} values. Out of the suggested solutions to arrive at discriminatory dissolution method, 'solution 1' and 'solution 13' were selected for further evaluation and design validation. Figure 4 illustrates contour plots for all responses of solution 1, explaining the impact of selected variables. Response 1, 2, 3 and 4 were having moderate positive effect with increase in dissolution media volume and SLS concentration; Figure 4 (a) (b) (c) (d). Response 5 was having significant positive effect with increase in dissolution media volume which suggests that to achieve extent of release media volume shall be kept to the maximum possible; Figure 4 (e). Response 6 was not having any impact with the selected variables and was inversely proportional to agitation speed; Figure 4 (f). Contour plots of 'solution 13' are presented in Figure 5. Impact of selected variables on all responses were similar to that of 'solution 1'.

Graphical Optimization Criteria was used to produce an overlay graph. It was comprised of the contour plots from each response laid on top of each other. The numerical optimization criteria was carried over and automatically fills the Graphical Optimization criteria. Overlay plot of 'solution 1' is depicted in Figure 6 (a), white color defines the acceptable factor settings. Another color (Grey by default) defines the unacceptable factor settings. The color represented in white is termed as MODR, where all responses will meet the desired criteria by varying the factors in the allowable ranges. Overlay graph for 'solution 13' is depicted in

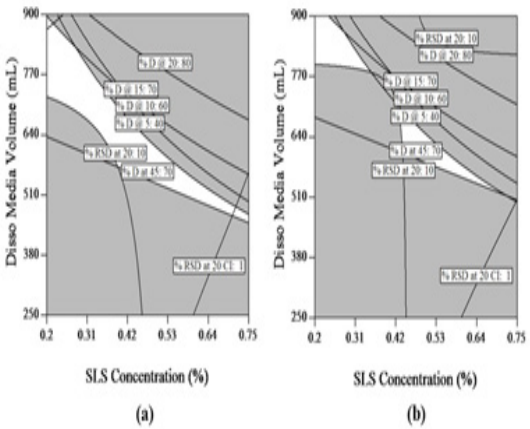


Figure 6: Overlay Plots showing the observed desirable space with input method parameters: concentration of SLS, dissolution medium volume, variable pH and variable speed. Here in % D at 5, 10,15,20,45 and % RSD at 20 were target responses for optimization. White colour field of chart defines the acceptable factor settings. Grey by default defines the unacceptable factor settings. The colour represented in white is termed as design space, where all responses will meet the desired criteria by varying the factors in the allowable ranges.

Table 6: Constraints for Optimisation and Solutions Prediction.

Name	Goal[a]	Lower Limit[b]	Upper Limit[b]	Importance
A	is in range	4.5	4.5	3
B	is in range	0.2	0.75	3
C	is in range	750	900	3
D	is in range	25	75	3
R1	minimize	10	35	3
R2	minimize	20	70	3
R3	minimize	30	80	3
R4	maximize	50	100	3
R5	maximize	75	110	3
R6	minimize	1	10	3

A, pH of dissolution media; B, sodium lauryl sulphate concentration (%w/v); C, dissolution media volume (ml); D, agitation speed (RPM); R1, response-1:% dissolution @ 5; R2, response-2:% dissolution @ 10; R3, response-3:% dissolution @ 15; R4, response-4:% dissolution @ 20; R5, response-5: % dissolution @ 45; R6, Response-6: % RSD of dissolution values at 20.

[a] given criteria for variables and responses;

[b] lower and higher limits for variables and responses.

Table 7: Numerical Solutions Table as Derived from DoE.

Number	Variables				Responses[a]						
	A	B	C	D	R1	R2	R3	R4	R5	R6[b]	
1	4.5	0.200	750	58	35	50	62	67	77	9.7	Selected
2	4.5	0.200	753	58	35	50	63	67	77	9.7	
3	4.5	0.200	759	57	35	50	63	67	77	9.7	
4	4.5	0.200	765	57	35	50	63	67	78	9.7	
5	4.5	0.203	750	58	35	50	62	67	77	9.7	
6	4.5	0.200	771	57	35	50	63	67	78	9.7	
7	4.5	0.200	774	56	35	50	63	67	78	9.8	
8	4.5	0.204	750	58	35	50	62	67	77	9.8	
9	4.5	0.200	778	56	35	50	63	67	78	9.8	
10	4.5	0.200	790	55	35	51	63	68	78	9.8	
11	4.5	0.211	750	57	35	50	62	67	77	9.8	
12	4.5	0.214	750	57	35	51	62	67	77	9.8	
13	4.5	0.200	802	54	35	51	63	68	78	9.9	Selected
14	4.5	0.200	807	54	35	51	63	68	78	9.9	
15	4.5	0.200	814	54	35	51	63	68	79	10.0	
16	4.5	0.200	821	53	35	51	64	68	79	10.0	

A, pH of media; B, sodium lauryl sulphate concentration(%w/v); C, dissolution media volume(ml); D, agitation speed (RPM); R1, response-1: % dissolution @ 5 min; R2, response-2: % dissolution @ 10 min; R3, response-3: % dissolution @ 15 min; R4, response-4: % dissolution @ 20 min; R5, response-5: % dissolution @ 45 min; R6, response-6: % RSD of dissolution values at 20 min.

[a] percentage(%) label claim.

[b] percentage(%) relative standard deviation.

Table 8: DoE Model Validation Table for Selected Solution.

Time (min)	A	B	C	D
5	34	34	35	36
10	50	61	51	60
15	62	74	63	71
20	67	85	67	79
45	77	93	78	88

A, 'Solution 1' prediction (pH 4.5, 0.2% SLS) 750ml, 60 RPM; B, ezetimibe tablets actual dissolution data from lab experiment (pH 4.5 acetate buffer 0.2% SLS) 750ml, 60 RPM; C, 'Solution 13' prediction (pH 4.5 acetate buffer, 0.2% SLS) 800ml, 55 RPM; D, Ezetimibe tablets actual dissolution data from lab experiment (pH 4.5 acetate buffer, 0.2% SLS) 800ml, 55 RPM.

Figure 6 (b) and has two design spaces to achieve the desired dissolution profile.

As predicted by the model, 'solution-1' and 'solution-13' were selected out of the 16 solutions suggested. These two solutions were executed in the lab with suggested combination of variables and dissolution profile was established. The established dissolution profile was compared against the DOE suggested solutions; Table 8. It was observed that the DOE suggested dissolution profiles were closely matching with the experimental results. Hence it is affirmed that the DOE model is validated and any of the two selected solutions can be finalized as suitable discriminatory dissolution method.

CONCLUSION

Discriminatory dissolution methods have a critical role in pharmaceutical tablet dosage form development. In generic formulations of solid oral dosage forms the success rate of *in-vivo* bioequivalence studies depends on the adequacy of discriminatory dissolution employed to study sameness of test and reference product. Poorly soluble molecules pose a great developmental challenge for dissolution method development. Developing a discriminatory dissolution method involves multiple variable that were interrelated, which makes the method development process cumbersome. DoE is the tried and tested approach in pharmaceutical industry and recommended by regulatory agencies for better scientific understanding of product and processes. DoE in dissolution method development can help accelerate the dissolution method development process to arrive at better discriminatory method with minimum runs. Poorly soluble molecule, ezetimibe was selected to demonstrate applications of DoE in dissolution method development. A simple 2-level factorial design was employed with 4 variables with center points and 6 responses, by analyzing dissolution profile at different time intervals. Model was evaluated for adequacy and statistical significance and solutions for discriminatory dissolution method were predicted with input criteria and numerical optimization criteria. Graphical evaluation tools like perturbation plots, contour plots, 3D surface plots and overlay plots help in better understanding of effects of variables on responses. Selected dissolution method conditions were experimented in lab with test product to establish dissolution profile values, which were compared against DoE predicted solutions. Experimental results were in agreement with predicted solutions which confirms design validation and achieved dissolution profile has discriminatory power with slow initial release profile and complete release of drug at 45 min (90%). By extending these concepts to pharmaceutical solid oral dosage forms one can develop discriminatory dissolution methods with minimal experimental runs, for poorly soluble molecules, which will further enhance the success rate of BE studies for generic products.

ACKNOWLEDGEMENT

Authors are thankful to GVK Biosciences Pvt. Ltd, formulation division for providing research lab facilities.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

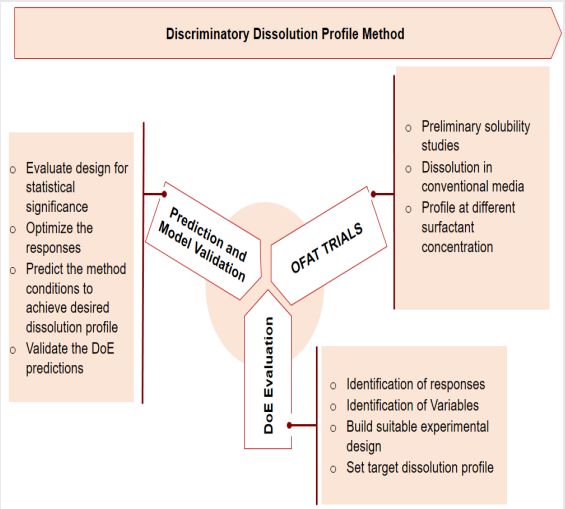
DOE: Design of Experiments; **SLS:** Sodium Lauryl Sulphate; **RPM:** Revolutions Per Minute; **HPLC:** High Performance Liquid Chromatography; **ANOVA:** Analysis of Variance; **FDS:** Fraction of Design Space; **MODR:** Method Operable Design Region.

REFERENCES

1. USFDA, Guidance for industry, Immediate Release Solid Oral Dosage Forms, Scale-up and Post approval Changes: Chemistry, Manufacturing and Controls, *in vitro* Dissolution Testing and *in vivo* Bioequivalence Documentation, Center for Drug Evaluation and Research (CDER). CMC. 1995;5.
2. Phil JB, Marion JC, Ivana D, Patrick J. Design and analysis of method equivalence studies. Analytical Chemistry. 2009;81(24):9849-57.
3. Marion JC, Phil JB. Acceptance criteria for method equivalency assessments. Analytical Chemistry. 2009;81(24):9841-8.
4. USFDA, Guidance for industry, Bioavailability and Bioequivalence Studies Submitted in NDAs or INDs, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Bio Pharmaceutics. 2014.
5. USFDA, Dissolution Testing of Immediate Release Solid Oral Dosage Forms, U.S. Department of Health and Human Services, Food and Drug

Administration, Center for Drug Evaluation and Research (CDER). BP. 1997;1.
6. USFDA, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products- General Considerations, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). 2003.
7. USP 40- NF 35, General Chapter, The Dissolution procedure: Development and Validation <1092>, The United States Pharmacopeial Convention. 1296-316.
8. USP 40- NF 35, General Chapter, Dissolution <711>, the United States Pharmacopeial Convention. 588-98.
9. USFDA, Guidance for industry, Bio equivalence recommendations of Ezetimibe tablets, U.S. Department of Health and Human Services, Food and Drug Administration, Office of Generic Drugs. 2008.
10. Dickinson PA, Lee WW, Stott PW, Townsend AI, Smart JP, Ghahramani P, *et al*. Clinical relevance of dissolution testing in quality by design. The AAPS Journal. 2008;10(2):280-90.
11. Phil JB, Marion JC, Phil N, Duncan T, Keith T. The application of quality by design to analytical methods. Pharmaceutical Technology. 2007;31(10):142-52.
12. Mark S, Matthias P, Hanna-Brown M, Phil N, Phil JB, Gordon H, *et al*. Implications and opportunities of applying QbD principles to analytical measurements. Pharmaceutical Technology. 2010;34(2):52-9.
13. Limin Z, John F, Hui Z, Harshad P, Scott J. Dissolution Method Development for Fixed-Dose Combination Drug Products – Challenges and Strategies. American Pharmaceutical review. 2015.
14. Kincl M, Turk S, Vrecer F. Application of experimental design methodology in development and optimization of drug release method. International Journal of Pharmceutics. 2005;291(1-2):39-49.
15. Vayeda CM, Devendra SY, Hitesh AJ. Quality by design based dissolution test development of Omeprazole extended release formulation. Asian Journal of Pharmaceutical and Clinical Research. 2014;7(5):150-4.
16. ZETIA (EZETIMIBE) tablets, Patient information leaflet (PIL), Merck/Schering-Plough pharmaceuticals, 29480958T, REV 14.
17. USFDA, Dissolution methods database, Ezetimibe tablets, U.S. Department of Health and Human Services, Office of generic drugs, Center for Drug Evaluation and Research (CDER). 2008.
18. USP 40- NF 35, Official monograph, Ezetimibe Tablets, the United States pharmacopeial convention. 8289-90.
19. Deepthi KN, Abbaraju PL, Ajay BK, Narandra JR. Formulation and *in-vitro* evaluation of conventional tablets of Ezetimibe by using solid dispersion. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;5(2):331-5.
20. Lalit K, Sreenivasa MR, Renuka SM, Girish KP. Full factorial design for optimization, development and validation of HPLC method to determine valsartan in nanoparticles. Saudi Pharmaceutical Journal. 2015;23(5):549-55.
21. Moolchand K, Sanjay K, Bhupinder S, Saranjit S. Implementation of design of experiments for optimization of forced degradation conditions and development of a stability-indicating method for furosemide. Journal of Pharmaceutical and Biomedical Analysis. 2014;96:135-43.
22. Alice MFL, Jean CW, Eckers C, Borman PJ, Chatfield MJ. Investigation into the factors affecting accuracy of mass measurements on a time-of-flight mass spectrometer using Design of Experiment. Rapid Communications in Mass Spectrometry. 2007;21(4):529-35.
23. George O, Alice L, Patrick J, Phil B, Marion C. Reduced-method robustness testing of analytical methods driven by a risk-based approach. Pharmaceutical Technology. 2010;22(4):72-86.
24. Dennis A, Mikael N, Susanne O, Jörgen S, Olof S, Silke K, *et al.* A quality control method enhancement concept-continual improvement of regulatory approved QC methods. Journal of Pharmaceutical and Biomedical Analysis. 2016;129:273-81.
25. ICH, Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances (Q6A), International Conference on Harmonization of technical requirements for Registration of Pharmaceuticals for human use. 1999.
26. Kose OC, Esim O, Kurbanoglu S, Savaser A, Ozkan S, Ozkan Y. Development of a suitable dissolution method for the combined tablet formulation of Atorvastatin and Ezetimibe by RP-LC Method. Current Drug Delivery. 2016;13(3):1-9.
27. USFDA, Quality by design for ANDAs: An example for immediate-release dosage forms, Example QbD IR Tablet, Module 3 Quality, 3.2.P.2 Pharmaceutical Development, Office of Generic drugs. 2012.
28. Shengyun D, Bing X, Gan L, Jianyu L, Zhong X, Xinyuan S, *et al.* Application of design of experiment and design space (DOE-DS) methodology for the HPLC Separation of Panax Notoginseng Saponins. The Open Chemical Engineering Journal. 2015;9:47-52.
29. Phil JB, John R, Chris J, Hann-Brown M, Roman S, Simon B. The development phase of an LC method using QbD principles. Journal of Separation Science. 2010;2:2-8.
30. EMA-FDA pilot program for parallel assessment of Quality-by-Design applications: lessons learnt and Q and A resulting from the first parallel assessment. EMA/430501/2013. 2013.

PICTORIAL ABSTRACT



SUMMARY

- Discriminatory dissolution method for poorly soluble Ezetimibe tablets is developed with the help of 2-Level factorial design. Design of Experiments software is used make simulations of different variables. With the help of statistical software's desired dissolution method conditions to achieve discriminatory profiles was predicted. Arrived dissolution method is validated against experimental runs to confirm DoE predictions.

ABOUT AUTHORS

Mr. Sudheer Babu Kolla: Is a graduate in pharmaceutical technology and working in renowned pharmaceutical organizations in the field of analytical research and development. Expertise areas include analytical method development, validation, method transfer, quality control and quality assurance of finished pharmaceutical dosage forms. Current research interests are in application of multivariate statistical methods for pharmaceutical quality control analytical techniques in dynamic method development.

Cite this article: Kolla SB, Vallabhaneni MR, Puttagunta SB, Venkata MS. Design of Experiments Approach to Discriminatory Dissolution Method Development of Poorly Soluble Drug in Immediate Release Dosage Form. Indian J of Pharmaceutical Education and Research. 2019;53(3):435-45.