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APPLICATION OF PLACKETT–BURMAN DESIGN OF EXPERIMENTS IN THE IDENTIFICATION OF "MAIN FACTORS" IN THE FORMULATION OF DABIGATRAN ETEXILATE MESYLATE IMMEDIATE-RELEASE TABLETS

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ABSTRACT: The current research paper was focused on exploiting Plackett-Burman design to screen the effect of eight factors Pregelatinised Starch (A), Crospovidone (B), Microcrystalline Cellulose PH 101 (C), Talc (D), Magnesium Stearate (E), Hydroxy Propyl Methyl Cellulose (F), Hydroxy Propyl Cellulose (G) and Lactose Monohydrate (H) on the release of drugs from the Immediate release DEM Cocrystal tablets prepared by direct compression method. The studies were carried out according to a statistical eight-factor 12 model using STATGRAPHICS XVI software and were subjected to a dissolution analysis at 0.1 N HCl pH 1.2 for determination of drug release. The ANOVA and the residual analysis showed the importance of the model. Pareto charts were generated using Design Expert® for visual identification of the effect of excipients on drug release. The drug release percentage was significantly affected by Crospovidone, Hydroxy propylmethyl cellulose, and microcrystalline cellulose. Plackett Burman could guide the process of reducing the number of experiments to a manageable level rationally.

INTRODUCTION: In order to systematically analyze formulation/process variables and link them to essential product quality attributes, conventional pharmaceutical product production uses factorial (full and/or fractional) statistical designs and response surface methodologies [1, 2]. These designs provide a detailed understanding of the process and are invaluable in determining the production process and the factors affecting the quality of the end product.

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Statistical methodologies, however, suffer from the practical constraint that many more experiments must be carried out with each variable added to the analysis, and one can easily establish a situation in which the number of experiments required to complete an experimental design is not technically feasible [3].

This is a challenge, especially in the early stages of growth where the formulation and/or process are not fixed, and many sources of variability exist, from the API, excipients as well as those arising from each unit operation of the manufacturing process. The underlying issue is that the analysis of too many variables directly raises the cost of production and takes more time to market new drugs, which may potentially hinder new

medications for patients with life-threatening diseases. In addition, limited market sizes have many unmet clinical needs and if it is too expensive to produce medication, then no new treatments will be forthcoming. Alternatively, the risk of inadequate process understanding is that studying too few variables will increase the possibility of product failures or recalls and safety concerns due to poor product efficiency, which also poses some risk to patients [4].

The use of risk methods to prioritize the variables to be studied is one way to maximize the use or resources in production, *i.e.*, to recognize the variables that present the most risk to the quality of the product and to study those variables more carefully. The purpose of this paper is to demonstrate how tools for quality risk management can be used to rationally balance the fine line between too many and too few experiments during product creation and to concentrate resources on the factors that can have the greatest effect on patient health/product quality [5]. One or more selected experimental responses are reported during this critical stage in Design of experiment (DOE) for a series of experiments carried out in a systematic way to establish a mathematical model.

These approaches include the postulation, for each response, of an empirical mathematical model that adequately represents a shift in response within the zone of interest. Instead of directly estimating the effects of each variable, response surface methodology (RSM) involves fitting the coefficients into a specific response variable's model equation and projecting the response in the form of a surface over the entire experimental domain [6]. RSM is mainly a group of statistical techniques for the creation of analytical models and model exploitation. It seeks to connect response to a number of predictors affecting it through careful design and analysis of experiments by generating a response surface, which is an area of space defined within the upper and lower limits of the independent variables representing the relationship between these variables and the measured response. Specifically, the objective is to apply quality risk management to the production of an Immediate release (IR) tablet for the poorly soluble drug Dabigatran Etexilate Mesylate (DEM) during the early stages of tablet formulation. In the early stages of development, the identification of key factors (sources of variability) was achieved using the method followed by the Plackett-Burman (PB) experiment design (DOE) method [7].

MATERIALS AND METHODS:

Materials: Dipyridamole was obtained from Micro Advanced Research Center (Bangalore, India) as a gift sample. HPMC K4M and Microcrystalline Cellulose 101, Lactose Monohydrate, were received as a gift sample from colorcon Asia (Pvt.) Ltd, Mumbai, India. Talc and Magnesium Stearate was purchased from S.D Fine Chemicals, Mumbai, India.

Screening of Excipients by Placket Barman Screening Design: Plackett–Burman screening designs are fractional factorial designs that are used to identify the effects of a large number of factors that are likely to affect critical qualities of a formulation. Because PB designs are fractional factorial designs, the number of runs needed to investigate main effects is equal to 2n or multiples of 4, and so they can be used to identify critical factors with the least number of experimental runs, with a very good degree of accuracy [8]. PB design screens a large number of input factors and, at the same time, reduces the number of runs. They are therefore very useful when the aim is to identify factors or variables that can be fixed or eliminated in further investigations [9, 10].

A set of experiments using the PB screening design was adopted to prepare DEM cocrystals IR tablets. This design investigates every input factor and arranges them on the Pareto Chart based on the magnitude of its influence with positive or negative signs respectively (blue or grey color) [11]. The ‘t’ statistic is determined by estimating the standard effect of each input factor. The factor with bar extending beyond the vertical line on the Pareto chart shows significant influence at 95% confidence level. The factors show positive or negative sign on the Pareto chart reflecting increased or decreased effect respectively when moving from lowest to the highest level for the specific factor. The ANOVA results were used to determine the most influencing effects. The variables which were significant at 5% level ($P<0.05$) from the regression analysis were considered to have greater impact on responses.

The variables were correlated using the following polynomial equation with PB design [12, 13].

$$Y = A0 + A1X1 + A2X2 + A3X3 + A4X4 + \ldots\ldots\ldots\ldots + AnXn \quad \ldots\ldots\ldots\ldots\ldots\ldots 1)$$

Where, Y is the response, A0 is the arithmetic mean response, and A1, A2……An are the coefficients of the factors X1, X2 ……Xn.

A Plackett–Burman statistical screening design was performed for screening of high-risk factors. Total twelve experimental trials involving eight independent variables were generated using STATGRAPHICS XVI.

Pregelatinized Starch (A), Crospovidone (B), Microcrystalline Cellulose PH 101 (C), Talc (D), Magnesium Stearate (E), Hydroxy Propyl Methyl Cellulose (F), Hydroxy Propyl Cellulose (G), and Lactose Monohydrate (H) were selected as independent variables, and drug release was set as the response variable.

Batch No	Pattern	A	B	C	D	E	F	G	H
1	++++++++++ +	1	1	1	1	1	1	1	1
2	-+-+++---+-	-1	1	-1	1	1	1	-1	-1
3	--+-+++---+	-1	-1	1	-1	1	1	1	-1
4	+--+-+++---	1	-1	-1	1	-1	1	1	1
5	-+--+-+++--	-1	1	-1	-1	1	-1	1	1
6	--+--+-+++-	-1	-1	1	-1	-1	1	-1	1
7	---+--+-+++	-1	-1	-1	1	-1	-1	1	-1
8	+---+--+-++	1	-1	-1	-1	1	-1	-1	1
9	++---+--+-+	1	1	-1	-1	-1	1	-1	-1
10	+++---+--+-	1	1	1	-1	-1	-1	1	-1
11	-+++---+--+	-1	1	1	1	-1	-1	-1	1
12	+-+++---+--	1	-1	1	1	1	-1	-1	-1

Direct Compression and Tablet Production: IR tablets of DEM cocrystals were prepared by direct compression method using Crospovidone (CP) as super-disintegrant to improve the dissolution of drug. HPMC K4M was used as binder. Micro-crystalline cellulose (MCC) and lactose mono-hydrate were use as diluent, respectively and Talc is used as glidant. DEM cocrystals equivalent to 75 mg of DEM and all the excipients except magnesium stearate were taken in mortar. Then powder blend was mixed well for 15 to 30 min. The blends were passed through # 80 sieve. Lubrication was done using magnesium stearate. Final blend was compressed on Remake Mini Press II D Tooling 8 station compression machine equipped with concave punches to a weight of 300 mg/tablet. The compressed tablets were evaluated for pre and post-compression parameters.

***In-vitro* Drug Release for DEM Cocrystal Immediate Release Tablets:** The study was carried out using USP dissolution test apparatus II (DS 8000, Labindia, Mumbai, India) at 50 rpm in 900 ml of 0.1 N HCl (pH 1.2) as a dissolution media. The temperature was maintained at 37± 0.5 ºC. The samples were withdrawn at pre-determined time intervals of 0, 10, 20, 30, 45, 60 min. Aliquots (5 ml) were withdrawn, filtered, and analyzed spectrophotometrically using UV spectro-photo-meter (3000+, Labindia, Mumbai) at 235 nm. An equal amount of fresh dissolution medium, pre-warmed at 37±0.5ºC, was added after each sampling to maintain the sink condition throughout the study. A dissolution study was performed in triplicate for each batch. The kinetic study of drug release data was done by zero-order (cumulative percentage drug released *vs.* time) and first-order (percentage drug retained *vs.* time) plots [14, 15].

Statistical Methods: Normal plots were used to determine effect significance for the 12 run PB studies. For the normal plots, if the responses fall in line with the expected values from a normal distribution, then the effect was considered insignificant. Conversely, if responses fall out of line with the expected values, the effect was considered significant. To confirm the half-normal plots, t-tests and Pareto charts were generated using Design Expert® (version 8.0.4; Stat Ease, Inc., Minneapolis, MN); a significant threshold of p threshold of $p<0.05$ was used. The Pareto charts help to visualize the relative size of each effect.

RESULTS AND DISCUSSION:

Screening of Excipients by Placket Barman Screening Design: The PB design was applied as a screening method to identify the significant factors that most influence the preparation of the tablet dosage form either positive or negative impact [3, 4].

Predicting the primary effect of formulation and process parameters on responses is a critical requirement in the development of DEM Cocrystals immediate release tablets by direct compression method.

Eight factors that may affect the experimental response were selected as independent variables at two levels of the study, as shown in **Table 1**.

TABLE 1: EXPERIMENTAL VARIABLES AND LEVELS OF PB DESIGN

Independent Variables	Units	Low	High
A: Pregelatinised Starch	mg	3	9
B: Crosspovidone	mg	3	9
C: Microcrystalline Cellulose	mg	100	200
D: Talc	mg	1	5
E: Magnesium Stearate	mg	1	5
F: Hydroxy Propyl Methyl Cellulose	mg	6	12
G: Hydroxy Propyl Cellulose	mg	6	12
H: Lactose Monohydrate	mg	24	84

Pregelatinised starch (A), Crosspovidone (B), Microcrystalline Cellulose PH 101 (C), Talc (D), Magnesium Stearate (E), Hydroxypropyl Methyl Cellulose (F), Hydroxypropyl Cellulose (G) and Lactose Monohydrate (H) were selected as the independent variables and drug releases were established as the response variable.

A total of twelve experimental trials with independent variables were generated using STATGRAPHICS XVI. **Table 2** shows the pattern and observed responses of the two-level PB formulation (PBF).

TABLE 2: OUTLINE AND OBSERVED RESPONSES OF PB-FORMULATIONS

PBF No.	A	B	C	D	E	F	G	H	Drug Release
	mg	mg	mg	mg	mg	mg	mg	mg	%
1	9	9	100	5	5	6	12	24	84
2	3	3	100	5	5	12	6	84	45
3	3	9	100	1	1	12	12	84	76
4	9	3	100	1	5	12	12	24	40
5	9	9	200	1	5	12	6	84	90
6	9	3	200	5	1	12	6	24	50
7	3	9	200	1	5	6	6	24	90
8	9	3	200	1	1	6	12	84	50
9	9	9	100	5	1	6	6	84	80
10	3	3	200	5	5	6	12	84	69
11	3	9	200	5	1	12	12	24	79
12	3	3	100	1	1	6	6	24.0	80.0

Note: Pregelatinised Starch (A), Crosspovidone (B), Microcrystalline Cellulose PH 101 (C), Talc (D), Magnesium Stearate (E), Hydroxy Propyl Methyl Cellulose (F), Hydroxy Propyl Cellulose (G) and Lactose Monohydrate (H).

Effect of Independent Factors on Dissolution: The influence of independent factors on drug release is explained according to their order of classification. Drug release from the DEM co-crystal immediate release tablets was found to be in the range of 40-90% w/v depending on the polymer concentration **Table 3** [1, 6, 7].

TABLE 3: SUMMARY OF ANALYSIS OF VARIANCE

Factors	Drug Release	
	F-Ratio	P-Value
A: Pregelatinised Starch	0.92	0.4082
B: Crosspovidone	**12.37**	**0.0390**
C: Microcrystalline Cellulose	0.24	0.6576
D: Talc	0.16	0.7126
E: Magnesium Stearate	0.00	0.9530
F: Hydroxy Propyl Methyl Cellulose	2.42	0.2176
G: Hydroxy Propyl Cellulose	0.62	0.4879
H: Lactose Monohydrate	0.08	0.7997

The Pareto Chart indicated that the factor Crosspovidone (B) has a significant influence on drug release **Fig. 1**. The ANOVA results also confirmed that all of these factors have p-values less than 0.05, indicating that the factors are significantly different from zero at a 95.0% confidence level **Table 3**.

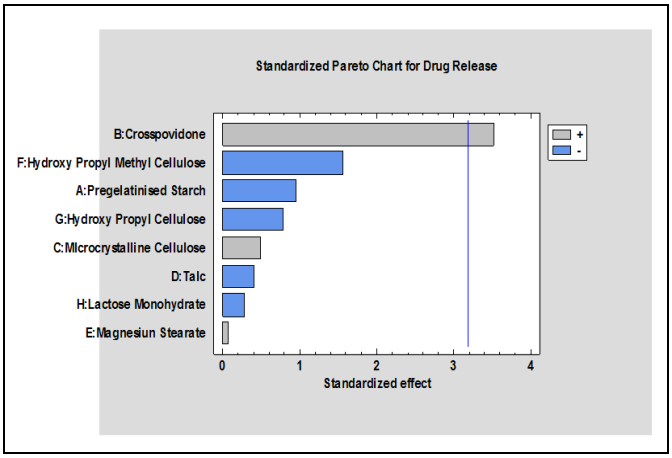

FIG. 1: PARETO CHART OF THE STANDARDIZED EFFECTS OF INDEPENDENT FACTORS ON DRUG RELEASE

The regression coefficient for drug release indicates variability of 97.42% around the mean. The main effect graph **Fig. 2** confirmed the direct relationship between the amount of Crosspovidone and drug release. Based on the above results, the input factor Crosspovidone was set to appropriate values for further optimization studies. The pronounced positive effect of Crosspovidone (p-0.0390) on drug release can be attributed to the composition of the water-insoluble synthetic cross-linked homo-polymer of N-vinyl-2-pyrrolidinone. Quickly exhibits high capillary activity and pronounced hydration capacity, to form a gel. The particle size of crospovidone strongly influences disintegrating tablets. The larger the particles, the faster the disintegration. Crospovidone can also be used as a solubility enhancer. The drug is adsorbed onto crospovidone in the presence of an appropriate solvent, and then the solvent is evaporated. This technique results in a faster dissolution rate. This study indicated that this could be the reason to show the positive effect of Crosspovidone on drug release [18, 19].

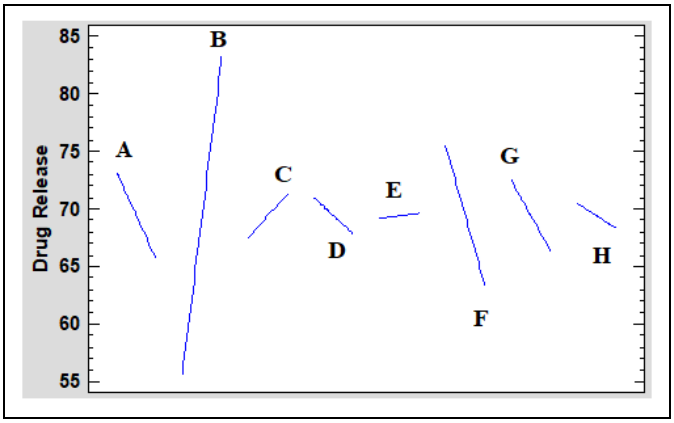

FIG. 2: MAIN EFFECT PLOT OF INDEPENDENT VARIABLES ON DRUG RELEASE

This study indicated that the increasing concentrations of hydroxypropyl methylcellulose may have contributed to delaying the drug release, which is reflected on the negative sign in the Pareto graph. Another variable microcrystalline cellulose showed negligible positive impact as a burden on drug release, as shown in the **Table 8.4**, **Fig. 8.10**. It can be concluded that crospovidone, hydroxyl-propyl methylcellulose and microcrystalline cellulose have shown an effect on drug release in the formation of immediate release tablets of stable DEM co-crystal and therefore were further chosen for the Box-Behnken design [16, 17, 20, 21].

Effect of (B) Crospovidone: The positive significant effect of crospovidone indicates a direct relationship with that of polymer. The Cros-povidone in contact with the media exhibits high capillary activity and pronounced hydration capacity with little tendency to form gels. The same mechanism might have occurred when tablet comes in contact with media [5].

Effect of (F) Hydroxypropyl Methyl Cellulose: HPMC K4M also showed a retarding effect on the drug release. Initially, the drug release from the increasing amount of hydrophilic polymer might have retarded the drug release at a particular concentration where the internal strength and viscosity of the polymer entanglement was more [5, 6].

Effect of (C) Microcrystalline Cellulose: Positive significant effect was shown by MCC because of apart from its diluents property, it has some disintegration property also [7, 8, 22, 23].

CONCLUSION: PB screening design was used to determine the significant main effects among these. Pareto charts and main effect chart obtained from Plackett Burman screening design depicted those formulation variables, Crosspovidone was found to be the most influencing variable. Also, Hydroxy-propyl Methylcellulose and Microcrystalline Cellulose showed an effect on drug release. Hence this factor can be considered for optimization of formulation of DEM cocrystals IR tablets with reducing the experimental trial.

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