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An optimization study of rifampicin oral suspension formulation using central composite design of experiment model

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Abstract
In the present study, an optimization design of experiments was applied in evaluation of oral suspension formulation of Rifampicin. Rifampicin is a widely used antibiotic to treat a several types of bacterial infections like tuberculosis, leprosy, and Legionnaire's disease. Central Composite Design optimization design was used to study main and interaction effect of variables affecting CQAs. Impact of formulation and manufacturing process variables like hydrocolloid (%) and homogenization speed (rpm) was studied on critical quality attributes (CQAs) of Rifampicin oral suspension. Viscosity (cps) and content uniformity (% C.V.) were identified as CQAs of Rifampicin oral suspension.

Keywords: QBD, DOE, Optimization, central composite design, analysis of variance, response surface design

Introduction

Rifampicin

Rifampicin, also known as rifampin, is an antibiotic used to treat a several types of bacterial infections [1]. This includes tuberculosis, leprosy, and Legionnaire's disease. It is almost always used along with other antibiotics, except when given to prevent Haemophilus influenzae type b and meningococcal disease in those who have been exposed to those bacteria. Before treating someone for a long period of time, measurement of liver enzymes and blood counts are recommended. It can be given either by mouth or intravenously [2].

Rifampicin was discovered in 1957 and first sold as a medication in 1971 [3, 4]. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system [5]. The wholesale cost in the developing world is about 3.90 USD a month [6]. In the United States it is expensive with a month of treatment being about 120 USD [2, 7]. Rifampicin is made from Amycolatopsis rifamycinica [4].

Medical Uses

Rifampicin is used for the treatment of tuberculosis in combination with other antibiotics, such as pyrazinamide, isoniazid, and ethambutol [8]. For the treatment of tuberculosis, it is administered daily for at least 6 months [9]. Combination therapy is utilized both to prevent the development of resistance and to shorten the length of treatment [10]. Resistance of Mycobacterium tuberculosis to rifampicin develops quickly when it is used without another antibiotic, with laboratory estimates of resistance rates from 10−7 to 10−10 per tuberculosis bacteria per generation [11, 12].

Rifampicin can be used alone in patients with latent tuberculosis infections to prevent the development of active disease because only small numbers of bacteria are present. A Cochrane review found no difference in efficacy between a three to four month regimen of rifampicin and a six-month regimen of isoniazid for preventing active tuberculosis in patients not infected with HIV, and patients who received rifampicin had a lower rate of hepatotoxicity [13]. However, the quality of the evidence was judged to be low [13]. A shorter two-month course of rifampicin and pyrazinamide had previously been recommended, but is no longer due to high rates of hepatotoxicity [14].

Rifampicin should be taken on an empty stomach with a glass of water. It is generally taken either at least one hour before meals or two hours after meals [15]. Rifampicin is also used to treat non-tuberculous mycobacterial infections including leprosy (Hansen's disease) and Mycobacterium kansasii [16].

With multidrug therapy used as the standard treatment of Hansen's disease, rifampicin is always used in combination with dapsone and clofazimine to avoid causing drug resistance.

Pharmaceutical Suspension

A Pharmaceutical Suspension is a two- phase system with uniform dispersion of finely divided solid drug particles in a continuous phase of solid, liquid or gas in which the drug has minimum solubility. Here in suspensions, the finely divided solid drug particles are called as dispersed phase or external phase or discontinuous phase and the phase in which they are dispersed is called as dispersion medium or internal phase or continuous phase [17].

Suspensions offer distinct advantages mentioned below:

1. Stability: Some drugs are not stable in solution form. In such cases it is necessary to prepare an insoluble form of that drug. Therefore drugs are administered in the form of suspension. e.g. Procaine Penicillin G.

2. Choice of solvent: If the drug is not soluble in water and solvents other than water are not acceptable, suspension is the only choice. e.g. Parenteral corticosteroid.

3. Mask the Taste: In some cases drugs are made insoluble and dispensed in the form of suspension to mask the objectionable taste. e.g. Chloramphenicol base is very bitter in taste, hence the insoluble chloramphenicol palmitate is used which does not have the bitter taste

4. Prolonged Action: Suspension has a sustaining effect, because, before absorption the solid particles should be dissolved. This takes some time. e.g. Protamine Zinc Insulin and procaine penicillin G.

5. Bioavailability: Drugs in suspension exhibit a higher bioavailability compared to other dosage forms (except solution) due to its large surface area, higher dissolution rate. e.g. Antacid suspensions provides immediate relief from hyperacidity than its tablet chewable tablet form.

A Central composite design of experiments

A central composite design is the most commonly used response surface design experiment. Central composite designs are a factorial or fractional factorial design with center points, augmented with a group of axial points (also called star points) that help to estimate curvature.

A central composite design can be used to efficiently estimate first- and second-order terms. Model a response variable with curvature by adding center and axial points to a previously-done factorial design.

Central composite designs are especially useful in sequential experiments because you can often build on previous factorial experiments by adding axial and centre points.

For example, to determine the best conditions for injection-molding a plastic part. One will first run a factorial experiment and determine the significant factors: temperature (levels set at 190° and 210°) and pressure (levels set at 50MPa and 100MPa). If the factorial design detects curvature, one can use a response surface design experiment to determine the optimal settings for each factor. The design points for this experiment are below.

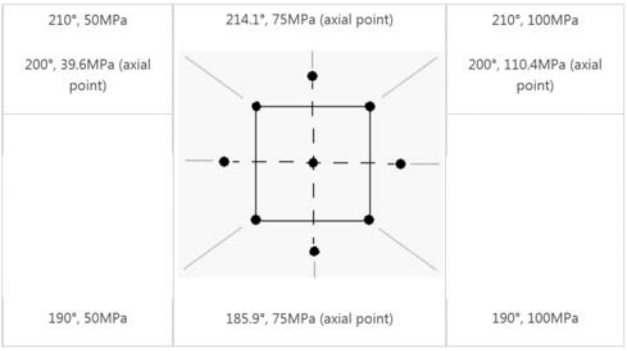

Fig 1: Points on the diagram represent the experimental runs that are done in central composite design of experiments

When possible, central composite designs have the desired properties of orthogonal blocks and rotatability.

Orthogonal blocks

Often, central composite designs are done in more than one block. Central composite designs can create orthogonal blocks, letting model terms and block effects be estimated independently and minimizing the variation in the regression coefficients.

Rotatability

Rotatable designs provide constant prediction variance at all points that are equidistant from the design center.

A face-centered central composite design

Face centered designs are a type of central composite design with an alpha of 1. In this design the axial points are at the center of each face of the factorial space, so levels = + 1. This variety of design requires 3 levels of each factor. Augmenting an existing factorial or resolution V design with appropriate axial points can also produce this design [13].

In the present study central composite design was applied for the optimization of Rifampicin oral suspension formulation. Impact of formulation and manufacturing process variables like hydrocolloid (%) and homogenization speed (rpm) was studied on critical quality attributes (CQAs) of Rifampicin oral suspension. Viscosity (cps) and content uniformity (% C.V.) were identified as CQAs of Rifampicin oral suspension.

Material & Methods

The materials used were obtained as gift samples from pharmaceutical company.

Preparation of Rifimipicin Oral Suspension

Powder blend of Rifampicin, sweetener, preservative, flavourant and Sodium CMC was prepared by conventional technique. All the ingredients were passed through 200# before mixing. Tween 80 was added to purified water. Then the powder blend was added to this surfactant containing purified water under homogenization. Different batches prepared at variable combination of factors X2 and X3 are shown in Table1.

Model Factor	Actual Values		Coded Values	
	Low	High	Low	High
X2: Hydrocolloid (%)	16.0	20.0	-1	+1
X3: Homogenization speed (rpm)	100.0	400.0	-1	+1

Table 1: Formulation Batches with Different Combination of Factors using Central Composite Design

Std Order	Run Order	Pt Type	Blocks	X2	X3
2	1	1	1	20.0	100.0
11	2	0	1	18.0	250.0
1	3	1	1	16.0	100.0
4	4	1	1	20.0	400.0
9	5	0	1	18.0	250.0
12	6	0	1	18.0	250.0
7	7	-1	1	18.0	37.9
13	8	0	1	18.0	250.0
6	9	-1	1	20.8	250.0
8	10	-1	1	18.0	462.1
3	11	1	1	16.0	400.0
10	12	0	1	18.0	250.0
5	13	-1	1	15.2	250.0

The prepared batches were analyzed for determining viscosity and content uniformity. Viscosity (cps) and content uniformity (% C.V.) were identified as CQAs of Rifampicin oral suspension.

Results & Discussion

Effect of different factor combinations on CQAs Viscosity (cps) and content uniformity (% C.V.) of Rifampicin oral suspension is shown in Table 2

Table 2: CQAs of Formulation Batches Prepared with Different Combination of Factors using Central Composite Design

Run Order	Pt Type	Blocks	X2	X3	Y1	Y2
1	1	1	20.0	100.0	540.0	6.1
2	0	1	18.0	250.0	478.0	5.4
3	1	1	16.0	100.0	570.0	4.5
4	1	1	20.0	400.0	580.0	4.8
5	0	1	18.0	250.0	460.0	8.5
6	0	1	18.0	250.0	620.0	6.5
7	-1	1	18.0	37.9	370.0	7.5
8	0	1	18.0	250.0	490.0	4.2
9	-1	1	20.8	250.0	520.0	4.5
10	-1	1	18.0	462.1	510.0	9.1
11	1	1	16.0	400.0	550.0	8.5
12	0	1	18.0	250.0	580.0	7.5
13	-1	1	15.2	250.0	590.0	9.5

Optimisation study central composite design

Optimisation study to examine effects and interactions of significant factors on product quality attributes mainly drug release. The optimisation study typically can use one of the following experimental designs; factorial, fractional factorial, central composite, mixture design, D-optimal, or Box-Behnken design. Central composite design was specifically selected for this study [13].

Summary of results of statistical analysis and optimization of the formulations using central composite design is given in Table 3. After a regression analysis for each of the responses the polynomial model established as follows:

$$Y = b_0 + b_1X_2 + b_2X_3 + b_{12}X_2X_3 + b_{11}X_2^2 + b_{22}X_3^2$$

where Y is the response, X_2–X_3 are the main effects of factors, X_2X_3 is the interaction effects of factors, X_2^2, $X_3^2$are quadratic effects of factors, b_0 is the constant, and b_1-b_2 are the coefficients of the factors. The p values of the regression coefficients (b_1–b_2) were determined to evaluate the significance of the factors on the responses. ANOVA was also applied to determine the significance of the model.

The obtained data was statistically analyzed using Central Composite DOE using Minitab Software version 14. Analysis Results are shown in Table 3.

Table 3: Analysis of Data using Central Composite DOE

Central Composite Design
Factors: 2 Replicates: 1
Base runs: 13 Total runs: 13
Base blocks: 1 Total blocks: 1

Two-level factorial: Full factorial
Cube points: 4
Center points in cube: 5
Axial points: 4
Center points in axial: 0

Alpha: 1.41421

Design Table (randomized)

Run Blk A B
1 1 1.00000 -1.00000
2 1 0.00000 0.00000
3 1 -1.00000 -1.00000
4 1 1.00000 1.00000
5 1 0.00000 0.00000
6 1 0.00000 0.00000
7 1 0.00000 -1.41421
8 1 0.00000 0.00000
9 1 1.41421 0.00000
10 1 0.00000 1.41421
11 1 -1.00000 1.00000
12 1 0.00000 0.00000
13 1 -1.41421 0.00000

Response Surface Regression: Y1, Y2 versus X2, X3
Response Surface Regression: Y1 versus X2, X3
The analysis was done using coded units.
Estimated Regression Coefficients for Y1

Term Coef SE Coef T P
Constant 525.60 30.57 17.194 0.000
X2 -12.37 24.17 -0.512 0.624
X3 27.25 24.17 1.128 0.297
X2*X2 30.32 25.92 1.170 0.280
X3*X3 -27.18 25.92 -1.049 0.329
X2*X3 15.00 34.18 0.439 0.674

S = 68.35 R-Sq = 39.5% R-Sq(adj) = 0.0%

Analysis of Variance for Y1

Source DF Seq SS Adj SS Adj MS F P
Regression 5 21320.5 21320.5 4264.1 0.91 0.523
Linear 2 7164.9 7164.9 3582.5 0.77 0.500
Square 2 13255.5 13255.5 6627.8 1.42 0.304
Interaction 1 900.0 900.0 900.0 0.19 0.674
Residual Error 7 32704.8 32704.8 4672.1
Lack-of-Fit 3 12997.6 12997.6 4332.5 0.88 0.523
Pure Error 4 19707.2 19707.2 4926.8
Total 12 54025.2

Obs StdOrder Y1 Fit SE Fit Residual St Resid
1 2 540.000 474.127 54.038 65.873 1.57
2 11 478.000 525.600 30.568 -47.600 -0.78
3 1 570.000 528.876 54.038 41.124 0.98
4 4 580.000 558.624 54.038 21.376 0.51
5 9 460.000 525.600 30.568 -65.600 -1.07
6 12 620.000 525.600 30.568 94.400 1.54
7 7 370.000 432.714 54.038 -62.714 -1.50
8 13 490.000 525.600 30.568 -35.600 -0.58
9 6 520.000 568.750 54.038 -48.750 -1.16
10 8 510.000 509.786 54.038 0.214 0.01
11 3 550.000 553.373 54.038 -3.373 -0.08
12 10 580.000 525.600 30.568 54.400 0.89
13 5 590.000 603.750 54.038 -13.750 -0.33

Estimated Regression Coefficients for Y1 using data in uncoded units

Term Coef
Constant 3197.39
X2 -291.612
X3 -0.114453
X2*X2 7.58125
X3*X3 -0.00120778
X2*X3 0.0500000

Response Surface Regression: Y2 versus X2, X3
The analysis was done using coded units.
Estimated Regression Coefficients for Y2

Term Coef SE Coef T P
Constant 6.4200 0.7597 8.451 0.000
X2 -1.1489 0.6006 -1.913 0.097
X3 0.6178 0.6006 1.029 0.338
X2*X2 -0.1300 0.6440 -0.202 0.846
X3*X3 0.5200 0.6440 0.807 0.446
X2*X3 -1.3300 0.8493 -1.566 0.161

S = 1.699 R-Sq = 53.1% R-Sq(adj) = 19.6%
Analysis of Variance for Y2

Source DF Seq SS Adj SS Adj MS F P
Regression 5 22.847 22.847 4.569 1.58 0.280
Linear 2 13.613 13.613 6.807 2.36 0.165
Square 2 2.158 2.158 1.079 0.37 0.701
Interaction 1 7.076 7.076 7.076 2.45 0.161
Residual Error 7 20.199 20.199 2.886
Lack-of-Fit 3 8.731 8.731 2.910 1.02 0.474
Pure Error 4 11.468 11.468 2.867
Total 12 43.046

Obs StdOrder Y2 Fit SE Fit Residual St Resid
1 2 6.100 6.373 1.343 -0.273 -0.26
2 11 5.400 6.420 0.760 -1.020 -0.67
3 1 4.500 6.011 1.343 -1.511 -1.45
4 4 4.780 4.949 1.343 -0.169 -0.16
5 9 8.500 6.420 0.760 2.080 1.37
6 12 6.500 6.420 0.760 0.080 0.05
7 7 7.500 6.586 1.343 0.914 0.88
8 13 4.200 6.420 0.760 -2.220 -1.46
9 6 4.500 4.535 1.343 -0.035 -0.03
10 8 9.100 8.334 1.343 0.766 0.74
11 3 8.500 9.907 1.343 -1.407 -1.35
12 10 7.500 6.420 0.760 1.080 0.71
13 5 9.500 7.785 1.343 1.715 1.65

Estimated Regression Coefficients for Y2 using data in uncoded units

Term Coef
Constant -13.3053
X2 1.70389
X3 0.0723634
X2*X2 -0.0325000
X3*X3 2.31111E-05
X2*X3 -0.00443333

Summary of results of statistical analysis and optimization of the formulations using central composite design is given in Table 3, shows that the responses Viscosity (cps) and content uniformity (% C.V.) are not impacted significantly due to change in hydrocolloid (%) and homogenization speed (rpm). No interaction effect of factors X_2 and X_3 is observed on the responses Viscosity (cps) and content uniformity (% C.V.).

From the regression coefficient values given in table 3 and surface and contour plots shown in Figure 2a and 3a it can be inferred that factors X_2 has inverse effect on response Y_1 and Y2 From the regression coefficient values given in table 3 and surface and contour plots shown in Figure 2b and 3b it can be inferred that factors X3 have positive effect on response Y_2.

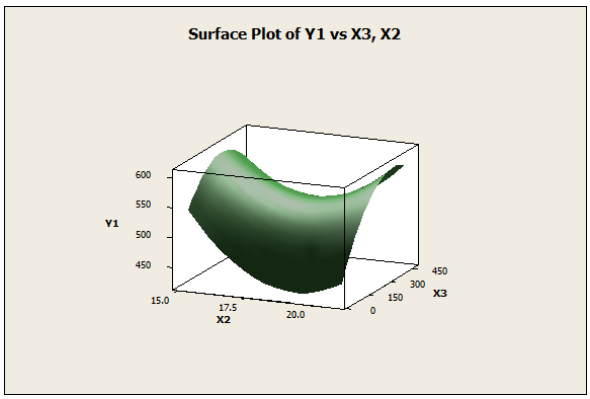

Fig 2a): Surface Plot of Y1 vs X3, X2

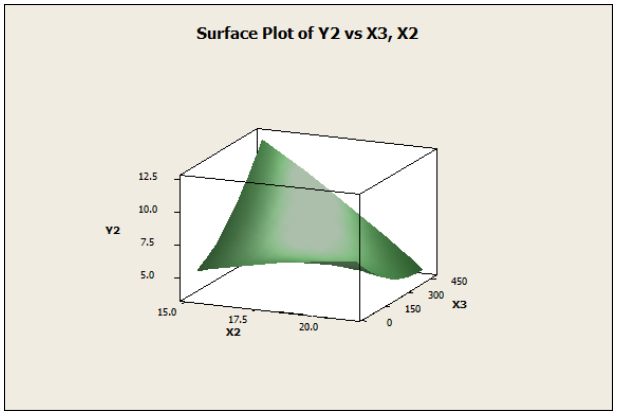

Fig 2b): Surface Plot of Y2 vs X3, X2

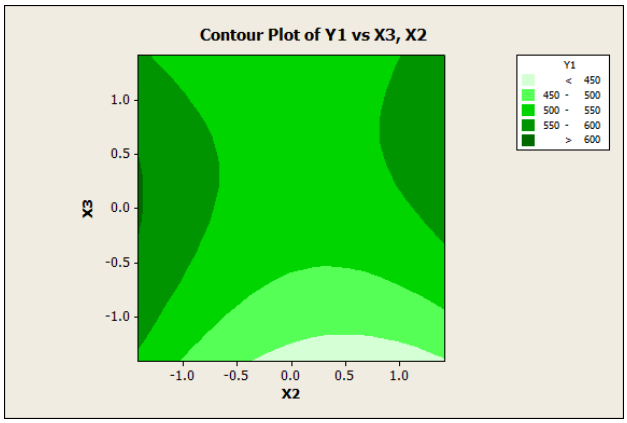

Fig 3a): Contour Plot of Y1 vs X3, X2

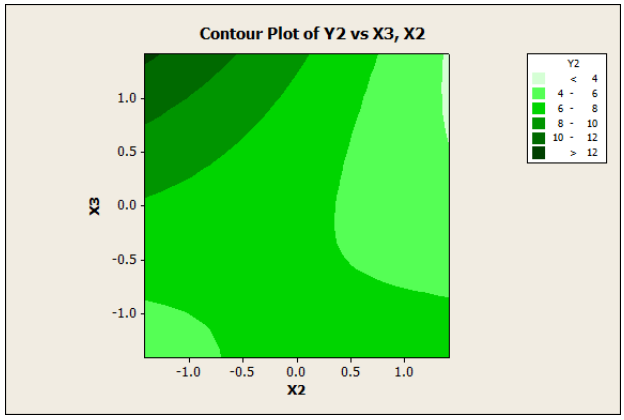

Fig 3b): Contour Plot of Y2 vs X3, X2

Evaluation of the design space

The design space for Rifampicin oral suspension formulation was established targeting the successful operating ranges for the responses drug Y1: Viscosity (cps) and Y2: Content uniformity (% C.V.) as 400-600% and 4-6% respectively. The proposed design space (Figure 4) comprising of the overlap region of ranges for the two responses was obtained. The design space demonstrates that the available operation range is wide at the laboratory scale and thus ensuring the product quality.

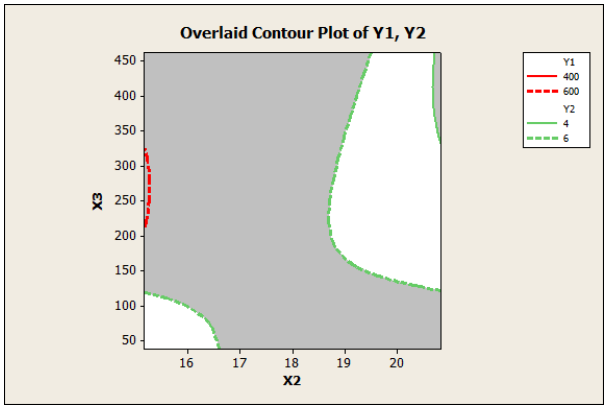

Fig 4: Overlaid Contour Plot of Y1, Y2

Conclusion

In the present study a central composite design was successfully applied for the optimisation of Rifampicin Oral Suspension Formulation. Optimisation study results revealed that hydrocolloid (%) and homogenization speed (rpm) do not have significant effect on Y1: Viscosity (cps) and Y2: Content uniformity (% C.V.) of given product. Using the design space plot obtained at the end of optimisation study one can select optimum amount of hydrocolloid (%) to achieve target Viscosity (cps) and Content uniformity (% C.V.). Thus it can be concluded that successful application of Central composite design of experiments is helpful to select optimum concentration of hydrocolloid (%) to reduce cost of raw materials which ultimately can improve profitability of pharmaceutical production unit. Also, manufacturing process with optimum homogenization speed (rpm) can help to improve durability of manufacturing equipment and subsequently reduce electricity consumption.

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