**International Journal of Pharmaceutical Science and Research**
**ISSN: 2455-4685, Impact Factor: RJIF 5.28**
**www.pharmacyresearchjournal.com**
**Volume 1; Issue 5; July 2016; Page No. 08-13**

# A screening study of rifampicin oral suspension formulation using plackett-burman design of experiment model

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**Abstract**
In the present study a screening design of experiments was applied in preliminary 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. A Plackett-Burman screening design was used to identify significant variables affecting critical quality attributes (CQAs). Impact of formulation and manufacturing process variables like surfactant (%), hydrocolloid (%), homogenization speed (rpm) and homogenization time (min) 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, Plackett-Burman, 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 Placket-Burman DOE**

These are the designs given in [18], up through n = 48, where n is the number of runs. In all cases except n = 28, the design can be specified by giving just the first column of the design matrix. In the table below, we give this first column (written as a row to save space). This column is permuted cyclically to get an (n - 1) x (n - 1) matrix. Then a last row of all minus signs is added. For n = 28, we start with the first 9 rows. These are then divided into 3 blocks of 9 columns each. Then the 3 blocks are permuted (rowwise) cyclically and a last column of all minus signs is added to get the full design.

Each design can have up to k = (n - 1) factors. If you specify a k that is less than (n - 1), just the first k columns are used.

12 Runs

+ + - + + + - - - + -

20 Runs

+ + - - + + + + - + - + - - - - + + -

24 Runs

+ + + + + - + - + + - - + + - - + - + - - - -

28 Runs

+ - + + + + - - - - + - - - + - - + + + - + - + + - +

+ + - + + + - - - - - + + - - + - - + - - - + + + - + + -

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32 Runs

- - - - + - + - + + + - + + - - - + + + + + + - - + + - + - - +

36 Runs

- + - + + + - - - + + + + + - + + + - - + - - - - + - + - + + + - - + -

40 Runs (note, derived by duplicating the 20 run design)

+ + - - + + + + - + - + - - - - + + - + + + - - + + + + - + - + - - - - + + -

44 Runs

+ + - - + - + - - + + + - + + + + + + - - - + - + + + - - - - - + - - - + + - + - + + + -

48 Runs

+ + + + + - + + + + + - - + - + - + + + + - - + + - - + + - + + + - - - + - + - + + - - - - + - - - -

**Material & Methods**

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

**Preparation of rifampicin 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 X1, X2, X3 and X4 are shown in Table 1.

| X1 | X2 | X3 | X4 |
|---|---|---|---|
| Surfactant (%) | Hydrocolloid (%) | Homogenization speed (rpm) | Homogenization time (min.) |

**Table 1:** Formulation Batches with Different Combination of Factors using Placket-Burman Screening Design

| Std Order | Run Order | Pt Type | Blocks | X1 | X2 | X3 | X4 |
|---|---|---|---|---|---|---|---|
| 2 | 1 | 1 | 1 | 10 | 20 | 100 | 120 |
| 8 | 2 | 1 | 1 | 6 | 16 | 400 | 120 |
| 3 | 3 | 1 | 1 | 6 | 20 | 400 | 80 |
| 7 | 4 | 1 | 1 | 6 | 20 | 400 | 120 |
| 12 | 5 | 1 | 1 | 6 | 16 | 100 | 80 |
| 6 | 6 | 1 | 1 | 10 | 20 | 400 | 80 |
| 5 | 7 | 1 | 1 | 10 | 20 | 100 | 120 |
| 1 | 8 | 1 | 1 | 10 | 16 | 400 | 80 |
| 4 | 9 | 1 | 1 | 10 | 16 | 400 | 120 |
| 10 | 10 | 1 | 1 | 10 | 16 | 100 | 80 |
| 11 | 11 | 1 | 1 | 6 | 20 | 100 | 80 |
| 9 | 12 | 1 | 1 | 6 | 16 | 100 | 120 |

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 Placket-Burman Screening Design

| Run Order | X1 | X2 | X3 | X4 | Y1 | Y2 |
|---|---|---|---|---|---|---|
| 1 | 10 | 20 | 100 | 120 | 558 | 7.8 |
| 2 | 6 | 16 | 400 | 120 | 487 | 4.5 |
| 3 | 6 | 20 | 400 | 80 | 578 | 4.2 |
| 4 | 6 | 20 | 400 | 120 | 589 | 4.3 |
| 5 | 6 | 16 | 100 | 80 | 499 | 8.1 |
| 6 | 10 | 20 | 400 | 80 | 601 | 4.1 |
| 7 | 10 | 20 | 100 | 120 | 590 | 8.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | 10 | 16 | 400 | 80 | 470 | 3.9 |
| 9 | 10 | 16 | 400 | 120 | 487 | 3.8 |
| 10 | 10 | 16 | 100 | 80 | 486 | 8.5 |
| 11 | 6 | 20 | 100 | 80 | 590 | 9.1 |
| 12 | 6 | 16 | 100 | 120 | 459 | 7.8 |

The obtained data was statistically analyzed using Placket-Burman screening DOE using Minitab Softyware version 14. Analysis Results are shown in Table 3.

**Table 3:** Analysis of Data Using Placket-Burman Screening Design

**Plackett - Burman Design**

| | | | |
|---|---|---|---|
| Factors | 4 | Replicates | 1 |
| Base runs | 12 | Total runs | 12 |
| Base blocks | 1 | Total blocks | 1 |

Design Table (randomized)

| Run | Blk | A | B | C | D |
|---|---|---|---|---|---|
| 1 | 1 | + | + | - | + |
| 2 | 1 | - | - | + | + |
| 3 | 1 | - | + | + | - |
| 4 | 1 | - | + | + | + |
| 5 | 1 | - | - | - | - |
| 6 | 1 | + | + | + | - |
| 7 | 1 | + | + | - | + |
| 8 | 1 | + | - | + | - |
| 9 | 1 | + | - | + | + |
| 10 | 1 | + | - | - | - |
| 11 | 1 | - | + | - | - |
| 12 | 1 | - | - | - | + |

Factorial Fit: Y1, Y2

Factorial Fit: Y1 versus X1, X2, X3, X4

Estimated Effects and Coefficients for Y1 (coded units)

| Term | Effect | Coef | SE Coef | T | P |
|---|---|---|---|---|---|
| Constant | | 532.833 | 4.624 | 115.22 | 0.000 |
| X1 | -1.667 | -0.833 | 4.624 | -0.18 | 0.862 |
| X2 | 103.000 | 51.500 | 4.624 | 11.14 | 0.000 |
| X3 | 5.000 | 2.500 | 4.624 | 0.54 | 0.606 |
| X4 | -9.000 | -4.500 | 4.624 | -0.97 | 0.363 |

S = 16.0193 R-Sq = 94.71% R-Sq(adj) = 91.69%

Analysis of Variance for Y1 (coded units)

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Main Effects | 4 | 32153.3 | 32153.3 | 8038.3 | 31.32 | 0.000 |
| Residual Error | 7 | 1796.3 | 1796.3 | 256.6 | | |
| Lack of Fit | 6 | 1284.3 | 1284.3 | 214.1 | 0.42 | 0.827 |
| Pure Error | 1 | 512.0 | 512.0 | 512.0 | | |
| Total | 11 | 33949.7 | | | | |

Estimated Coefficients for Y1 using data in uncoded units

| Term | Coef |
|---|---|
| Constant | 91.0000 |
| X1 | -0.41667 |
| X2 | 25.7500 |
| X3 | 0.0166667 |
| X4 | -0.225000 |

Least Squares Means for Y1

| | Mean | SE Mean |
|---|---|---|
| X1 | | |
| 6 | 533.7 | 6.540 |
| 10 | 532.0 | 6.540 |
| X2 | | |
| 16 | 481.3 | 6.540 |
| 20 | 584.3 | 6.540 |
| X3 | | |
| 100 | 530.3 | 6.540 |
| 400 | 535.3 | 6.540 |
| X4 | | |
| 80 | 537.3 | 6.540 |
| 120 | 528.3 | 6.540 |

Effects Plot for Y1

Effects Pareto for Y1

Factorial Fit: Y2 versus X1, X2, X3, X4

| Estimated Effects and Coefficients for Y2 (coded units) | | | | | |
|---|---|---|---|---|---|
| Term | Effect | Coef | SE Coef | T | P |
| Constant | | 6.192 | 0.1101 | 56.21 | 0.000 |
| X1 | -0.283 | -0.142 | 0.1101 | -1.29 | 0.239 |
| X2 | 0.183 | 0.092 | 0.1101 | 0.83 | 0.433 |
| X3 | -4.117 | -2.058 | 0.1101 | -18.69 | 0.000 |
| X4 | -0.250 | -0.125 | 0.1101 | -1.13 | 0.294 |

S = 0.381569 R-Sq = 98.05% R-Sq(adj) = 96.94%

Analysis of Variance for Y2 (coded units)

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Main Effects | 4 | 51.3700 | 51.3700 | 12.8425 | 88.21 | 0.000 |
| Residual Error | 7 | 1.0192 | 1.0192 | 0.1456 | | |
| Lack of Fit | 6 | 0.9392 | 0.9392 | 0.1565 | 1.96 | 0.498 |
| Pure Error | 1 | 0.0800 | 0.0800 | 0.0800 | | |
| Total | 11 | 52.3892 | | | | |

Estimated Coefficients for Y2 using data in uncoded units

| Term | Coef |
|---|---|
| Constant | 9.98889 |
| X1 | -0.0708333 |
| X2 | 0.0458333 |
| X3 | -0.0137222 |
| X4 | -0.00625000 |

Least Squares Means for Y2

| | Mean | SE Mean |
|---|---|---|
| X1 | | |
| 6 | 6.333 | 0.1558 |
| 10 | 6.050 | 0.1558 |
| X2 | | |
| 16 | 6.100 | 0.1558 |
| 20 | 6.283 | 0.1558 |
| X3 | | |
| 100 | 8.250 | 0.1558 |
| 400 | 4.133 | 0.1558 |
| X4 | | |
| 80 | 6.317 | 0.1558 |
| 120 | 6.067 | 0.1558 |

Pareto charts and residual plots showing effect of factors on CQAs are shown in Figure 1, 2, 3 and 4.

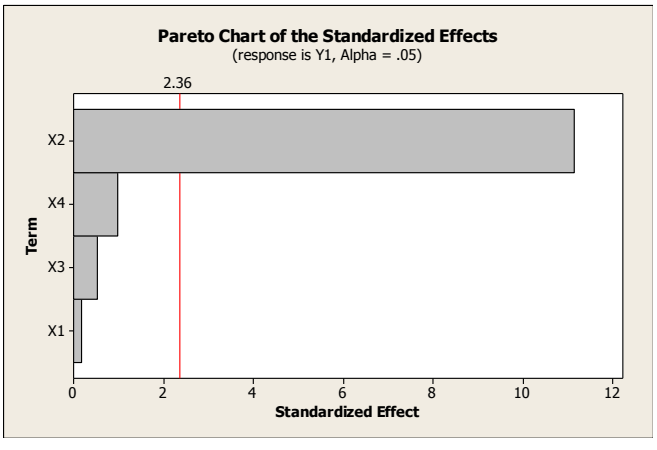


**Fig 1:** Pareto Chart showing Effect of X1, X2, X3 and X4 on Y1

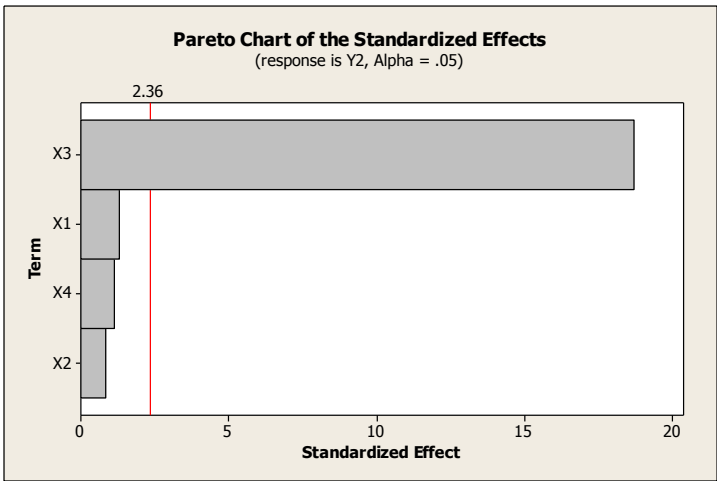


**Fig 2:** Pareto Chart showing Effect of X1, X2, X3 and X4 on Y2

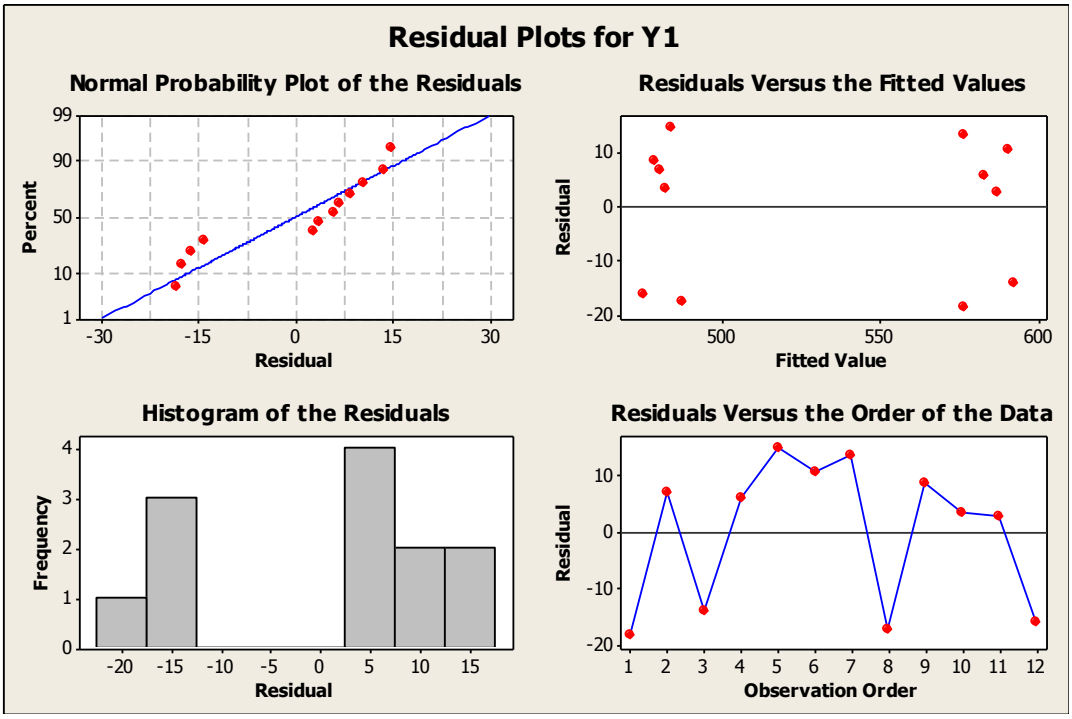


**Fig 3:** Residual Plots for Y1

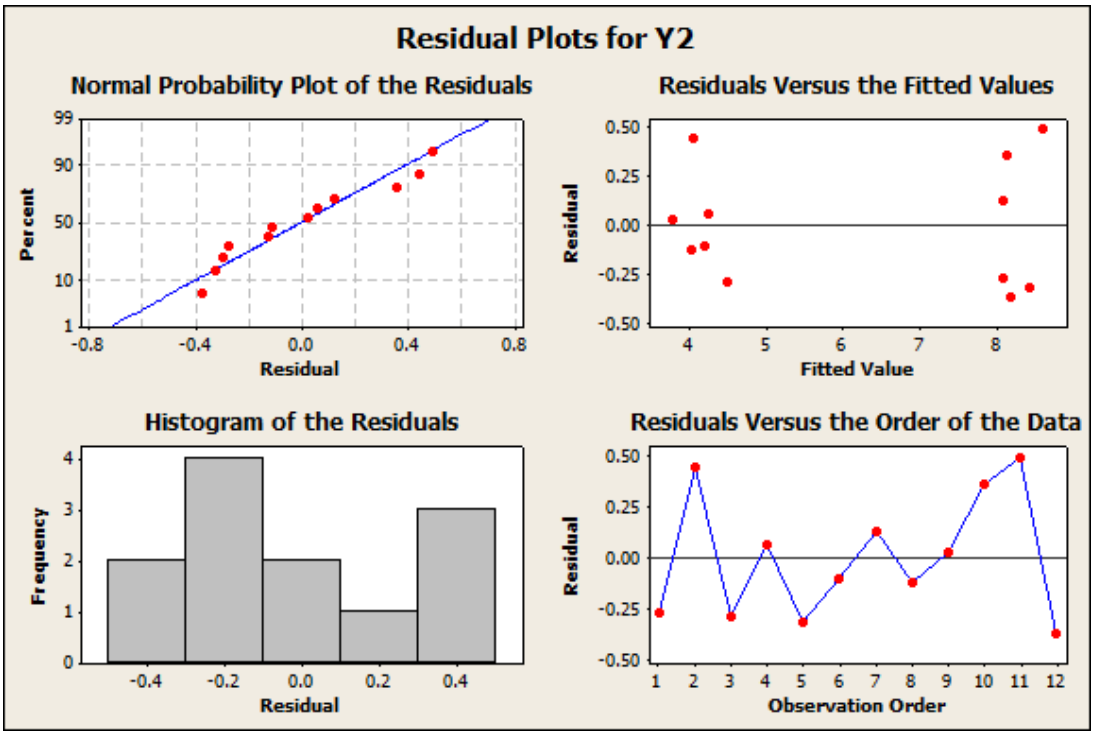


**Fig 4:** Residual Plots for Y2

From the Table 3 and Figure 1, 2, 3 and 4 it is observed that Y1 is significantly affected by factor X2 whereas Y2 is significantly affected by factor X3 ($p < 0.05$).

## Conclusion

In the present study a Placket-Burman screening design of experiments was applied successfully in preliminary evaluation of oral suspension formulation of Rifampicin. This study revealed that CQAs Viscosity (cps) and content uniformity (% C.V.) of Rifampicin oral suspension formulation are significantly affected by factors X2 Hydrocolloid (%) and X3 Homogenization speed (rpm). Main effect and interaction effect of these factors can be studied in optimization study. Thus, Placket-Burman screening design is useful to rule out non significant factors from significant ones so that optimization study will be carried out with fewer experimental runs. Thus, minimising cost of experiment by reducing consumption of material, machine and labour.

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