Chapter 5.3

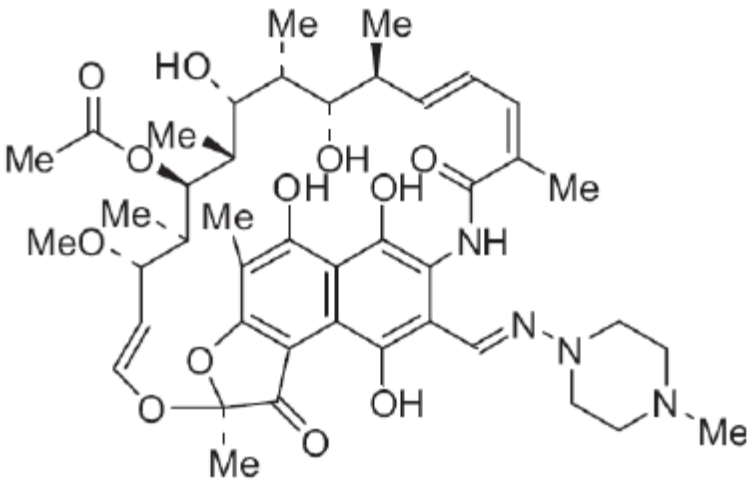
Quality by design enabled formulation development and optimization of floating sustained release Rifampicin tablet

5.3.1. Rifampicin-Drug Profile

Rifampicin (RIF) is a semisynthetic antibiotic derivative of rifamycin SV and is amongst frontline drugs used for the treatment of Tuberculosis.

General Characteristics: [1-5]

- **Molecular Formula:** $C_{43}H_{58}N_4O_{12}$
- **IUPAC Name:** 3-[[(4-Methyl-1-piperazinyl) imino]-methyl] rifamycin or 5,6,9,17,19,21-hexahydroxy-23-methoxy-2,4,12,16,20,22-heptamethyl-8-[N-(4-methyl-1-piperazinyl) formimidoyl]-2,7-(epoxypentadeca[1,11,13]trienimino) naphtho [2,1-*b*]furan-1,11(2H)-dione 21-acetate.
- **Structure:**



- **Molecular weight: 822.95**
- **Appearance and Color:** Red-brown crystalline powder.
- **Odor:** Odorless.
- **Solubility:** very slightly soluble in water at neutral pH, freely soluble in chloroform, soluble in ethyl acetate and in methanol.
- **Melting point:** 183 to 188^{o}C.
- **Dissociation constants:** Zwitterion with pKa 1.7 for the 4-hydroxy and pKa 7.9 for the 3-piperazine nitrogen.
- **Octanol/Water Partition Coefficient**: log Kow = 4.24.
- **Dose**: 150 mg and 300 mg.
- **pH of 1% suspension in water**: 4.5-6.5

Mechanism of action

RIF inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase [6]. It inhibits rpoB gene product β-subunit of DNA dependent RNA polymerase thereby acting in early transcription. Additionally, it is contemplated to bind to the ß-subunit, close to RNA/DNA channel and physically blocks the transit of the growing RNA chain. It physically blocks the formation of phosphodiester bond in the RNA backbone. [7, 8, 9].

Pharmacokinetics

RIF is well absorbed after oral administration reaching maximum peak plasma concentration at 1.5-3 hr over wide range of single dose. [5]. Absorption of RIF is reduced by about 30% when the drug is taken with food. RIF is widely distributed throughout the body it reaches in effective concentrations in many organs and body fluids which also include cerebrospinal fluid. [2]. Protein binding is around 84-91% [10].

The wide distribution is related to high lipotropism of RIF [5] and moreover as most of the unbound drug is not ionized, it readily diffuses into various tissues [2]. RIF elimination mainly occurs through bile and also through urine. The total elimination occurring through bile is not proportional to dose administered while elimination via urine increases with dose. RIF undergoes enterohepatic circulation and gets reabsorbed. The main metabolite excreted in urine and bile is 25-desacetylrifampin which is less lipophillic than RIF, does not get reabsorbed and easily excreted in urine [5].

Indications and Usage

Tuberculosis, leprosy and Meningococcal Carriers [11, 2].

Contraindications

RIF is contraindicated in known cases of hypersensitivity to the drug.

Drug Interactions

RIF is known to induce certain cytochrome P-450 enzymes mainly 3A4 isozyme [12]. It may accelerate the metabolism of drugs like phenytoin, quinidine, itraconazole, nifedipine, ciprofloxacin, nortryptiline, etc. [2].

Adverse Effects

Gastrointestinal like epigastric distress, anorexia, nausea, vomiting, diarrhoea, etc.; hematologic like thrombocytopenia; central nervous system like headache, fever, drowsiness, fatigue, mental confusion, etc. ocular, endocrine, renal, etc.[2].

5.3.2. Analytical methods

5.3.2.1. Determination of RIF by dual wavelength spectrophotometric method

Method described by Shishoo *et. al.* was used as it enables to determine RIF in presence of its major degradation product 3-Formyl rifamycin (3-FRSV). [13]. This method was used for estimation of RIF for assay and dissolution in initial screening and preliminary trials.

5.3.2.1.1. Preparation of standard stock solutions of RIF

RIF (100 mg) was dissolved in 30 ml of chloroform and volume was made up to 100 ml with chloroform to obtain stock solution of 1000 µg/ml. An aliquot of 10 ml was accurately taken out with graduated calibrated pipette and further diluted upto 100 ml with chloroform in to obtain working standard solution of 100 µg/ml.

5.3.2.1.2. Preparation of standard stock solution of 3-FRSV

3-FRSV (10 mg) was dissolved 30 ml of chloroform and volume was made up to 10 ml with chloroform to obtain stock solution of 1000 µg/ml. An aliquot of 1 ml was accurately taken out with graduated calibrated pipette and further diluted upto 10 ml with chloroform to obtain working standard solution of 100 µg/ml.

5.3.2.1.3. Preparation of calibration curve of RIF

Appropriate concentration of RIF and 3-FRSV from the working standard solution were prepared to obtain two different sets of dilutions as follows:

Set A: The series comprises of mixture of RIF and 3-FRSV having varying concentrations of RIF (5-50 µg/ml) and fixed concentration of 3-FRSV (5 µg/ml). The solutions of different concentrations were prepared from stock solution upon appropriate dilution with chloroform. The absorbances were measured at 475 nm and 507 nm and the difference between the two absorbances were plotted against concentration (µg/ml).

Set B: The series consisted of varying concentration of RIF (5-50 µg/ml) prepared with appropriate dilutions of stock solution with chloroform. The absorbances were measured at 475 nm and 507 nm and the difference between the two absorbances were plotted against concentration (µg/ml).

Accuracy and precision were carried out as per ICH guidelines [14]. For accuracy measurements were taken in triplicate and for precision six determinants were measured. The results of accuracy and precision are depicted in Table 1 and Table 2 respectively. No interference of excipients was found at specified detection wavelength.

The reference spectrum of RIF is depicted in Fig. 1. The calibration curve of RIF in presence and absence of 3-FRSV with regression analysis equation and correlation coefficient is depicted in Fig. 2 and Fig. 3 respectively.

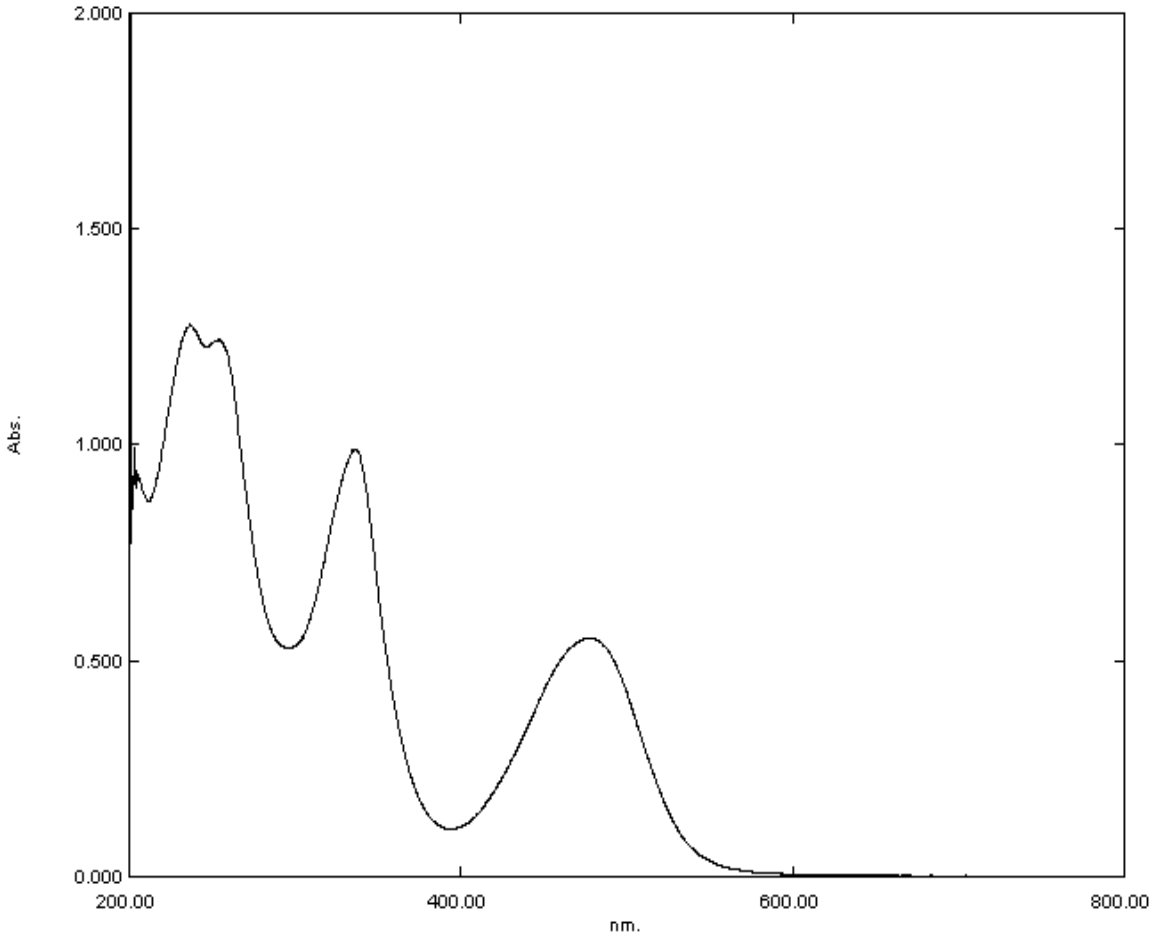


Fig. 1: Reference spectra of RIF in chloroform.

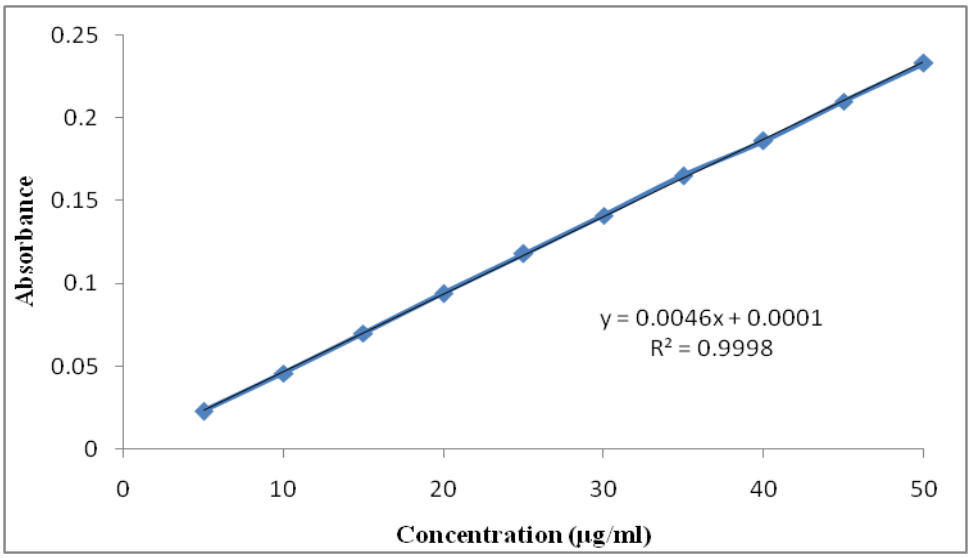


Fig. 2: Calibration curve of RIF in chloroform in presence of 3-FRSV.

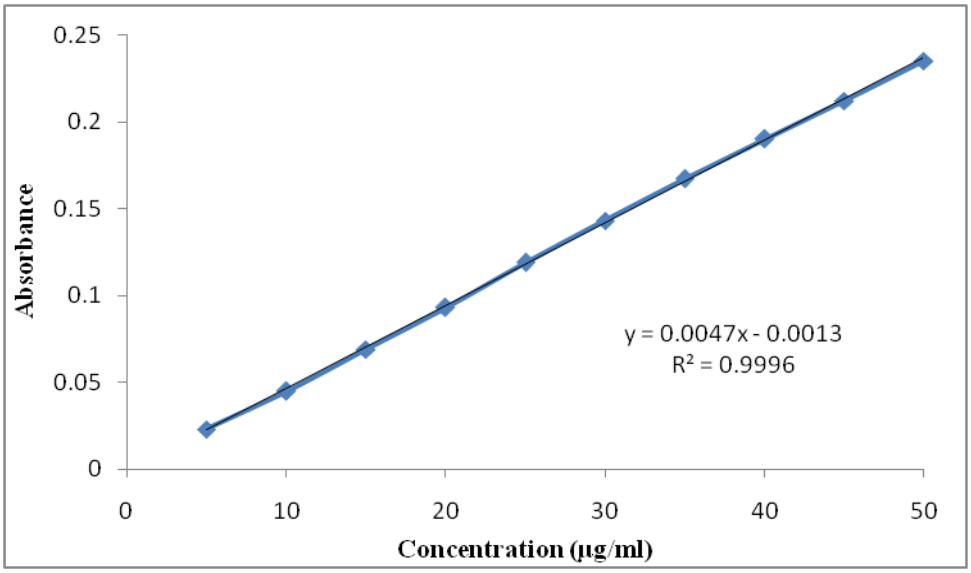


Fig. 3: Calibration curve of RIF in chloroform in absence of 3-FRSV.

% Recovery:

Table 1: % Recovery for RIF for dual wavelength method

Amount of Sample Taken Equivalent to (mg) Set A and Set B	Amount of Sample Spiked (mg) Set A and Set B	Amount of Spiked Sample Recovered Set A	% Recovery Set A	Amount of Spiked Sample Recovered Set B	% Recovery Set B
20	12	12.10±0.12	100.83	11.98±0.10	99.83
20	20	19.91±0.21	99.55	20.21±0.15	101.05
20	28	28.35±0.15	101.25	28.1±0.18	100.35

Data are represented as Mean± SD (n=3)

Precision:

Table 2: Precision for RIF for dual wavelength method

	% Relative Standard Deviation Set A	% Relative Standard Deviation Set B
Repeatability	0.98	0.85
Intraday	1.23	1.33
Interday	1.85	1.92

5.3.2.2. Determination of RIF by RP-HPLC method:

The development and validation of RP-HPLC method is discussed in detail in chapter 5.1.2.3. RP-HPLC method was used for estimation of RIF for assay and dissolution for optimization batches.

5.3.3. Methods

5.3.3.1. Quality Target Product Profile (QTPP) of RIF oral gastroretentive dosage form

The template for target product profile (TPP) has been provided by United States Food and Drug Administration (USFDA) guidance that portrays the parts of TPP for new drug applications [15]. The target product quality profile is enlisted as the quality properties that a drug product ought to possess so as to fulfill the objectives set in TPP as quantitative attributes [16]. The International conference of harmonization (ICH) Q8 (R2) [17] recapitulates them as QTPP. The QTPP lays the foundation of design criteria for the product and ought to embody patient relevant product performance characteristics. It should furnish a quantitative surrogate to ascertain the aspects of clinical safety and efficacy. Thus it ought to form the basis for determining the critical quality attributes (CQAs), critical material attributes, critical process parameters, and control strategy. The primary step in defining QTPP is to decide the type of dosage form, what is the purpose of your product, its key desired quality attributes, manufacturing methodology, etc. The anticipated QTPP depends upon scientific and nonscientific considerations [17, 18].

5.3.3.2. Risk assessment approach for identification of Critical Quality Attribute (CQA)

Risk based compliance is an imperative FDA initiative for current Good Manufacturing Practice for the 21st century [19]. ICH Q9 [20] guidance document introduced the concept of quality risk management for evaluating, communicating, controlling and reviewing risks to the quality of drugs across product life cycle. The ICH working definition of CQA was stated as: "A CQA is a quality attribute (a physical, chemical, biological or microbiological property or characteristic) that must be controlled (directly or indirectly) to ensure the product meets its intended stability, safety, efficacy and performance" [20]. The CQAs relies on the type of formulation, dosage form designed, manufacturing or production methodology, etc. employed and selected amongst many possible options. Consequently, formulation and process development typically rely on empirical prior knowledge and small scale feasibility studies. The identification of a CQA from the QTPP was based on the severity of harm caused by the product falling outside the acceptable range for that attribute.

5.3.3.3. Failure Mode and Effects Analysis (FMEA)

FMEA was initially developed outside health care system and its domain has now reached health care to assess risk of failure and harm in processes and to identify the most important areas for process improvements. FMEA is most efficient when it is performed before a design or process is established rather than after its implementation. It meticulously breaks down the analysis of complex processes into the manageable steps. An overall risk assessment of the drug product formulation components was performed to determine which formulation components have a high risk of impacting the drug product attributes.

The FMEA method was used to perform risk analysis, which could identify the failure modes that have the greatest chance of causing product failure, i.e., not meeting the QTPP. Using FMEA, the failure modes can be prioritized for a product or process for risk management purposes according to the seriousness of their consequences (effects), how frequently they occur and how easily they can be detected [18]. Thus FMEA is designed to assess the risk associated with failure modes, to rank the matters in terms of importance and to identify and carry out corrective actions to address the most serious concerns. The relative risk that each drug substance attributes presents was ranked according to risk priority number (RPN). Those attributes that could have a high impact on the drug product attributes needed to be studied in detail whereas those attributes that had low impact on the drug product attributes required no further investigation. The RPN was calculated with the Eq. 1 mentioned as below:

$$RPN = \begin{bmatrix}5\\4\\3\\2\\1\end{bmatrix} O \times \begin{bmatrix}5\\4\\3\\2\\1\end{bmatrix} S \times \begin{bmatrix}1\\2\\3\\4\\5\end{bmatrix} D \qquad (1)$$

where O is the occurrence, probability or the likelihood of an event to occur; it was ranked as 5, frequent; 4, probable; 3, occasional; 2, remote and 1, improbable to occur. The next parameter S is the severity, which is a measure of how severe of an effect a given failure mode would cause; it was ranked as 5, catastrophic; 4, critical; 3, serious; 2, minor and 1, negligible or no effect. The final parameter D is the detectability which means the ease that a failure mode can be detected. Thus the more detectable a failure mode is, the less risk it presents to product quality. For D, it was

ranked 1, absolute certain or easily detectable; 2, high detectable; 3 moderately detectable; 4, low or remote detectable and 5 as hard to detect or absolute uncertain.

5.3.3.4. Preparation of RIF floating tablet

Wax was selected to prepare sustain release tablets and the tablets were prepared by direct compression method. Briefly, accurately weighed quantity of RIF (150 mg/tab), microcrystalline cellulose (MCC PH 102), wax (glyceryl behenate, stearic acid, carnauba wax, glyceryl monostearate), pore former (HPMC E5/sodium chloride) and gas generating agent; sodium bicarbonate were sifted through ASTM # 30 (Jayant Scientific Industries, Mumbai, India) and physically mixed for about 10 min. Then silicon dioxide was sifted through ASTM sieve # 60 and added as a glidant to blend and blended for 5 min. Finally magnesium stearate was sifted through ASTM sieve 60# and added as lubricant and blended for 3 min. The homogeneous powder mixture were compressed on an eight station automatic rotary tablet machine (JM-8, General Machinery Co., Mumbai, India) equipped with flat faced beveled edges punches of 7.0 mm diameter to a target weight of 240 mg / tab. Thermal sintering of tablets were done by individually placing it on aluminum foil on a stainless steel tray which was further put into an oven (Shree Kailaish Industries, India) maintained at 80^0C. After 20 minutes, the tray was removed from the oven and tablets were allowed to cool to room temperature. Dissolution and physical tests were performed at least 24 hours after the treatment. From the preliminary experiments conducted, it was found that 20 minutes was sufficient for melting and redistribution of wax. Beyond this time tablet aspect ratio and dimensions were distorted. Hence, sintering time was fixed for all of the trials in order to exclude its effects on formulations.

5.3.3.5. Physical characterization of the tablets

The compressed tablets were subjected to variegated physical investigations like appearance, weight variation, hardness and drug content. The weight variation was carried out on 20 tablets using electronic balance (Shimadzu AX 120, Japan). Tablet hardness was determined prior to thermal treatment and at least after 24 hour of thermal treatment using minimum 6 tablets for each batch with dial type tablet hardness tester (Scientific Engineering Corporation, Delhi, India) respectively. Friability was determined by Friabilator (VFT-2D, Veego Instruments Co., Mumbai,

India) for 4 min at 25 rpm. Drug content measurement was done in triplicate using developed UV-Visible spectrophotometric method and RP-HPLC method as discussed in section 5.3.2.

5.3.3.6. *In vitro* buoyancy study

The *in vitro* buoyancy test was performed using United States Pharmacopeia (USP) 34 paddle type apparatus (VDA 6-DR,Veego Instruments Corporation, Mumbai, India) using 900 ml of 0.1 N hydrochloric acid (HCl) at rotation of 50 rpm at 37^0C ± 0.5^0C. Floating lag time was noted as the time interval between introduction of the tablet in the dissolution vessel to the time for tablet to the rise to surface of medium [21]. The duration of time the tablet constantly floated was considered as total floating time. Both the determinations were made in triplicate.

5.3.3.7. *In vitro* drug release study

Dissolution study was carried out in 900 ml 0.1N HCl in USP 34 dissolution apparatus II (VDA 6-DR,Veego Instruments Corporation, Mumbai, India) at 37±0.5^0C at 50 rpm [21]. Sample (10 ml) was withdrawn at 1, 2, 4, 6, and 8 hr and was replenished with equal volume of dissolution medium. Samples withdrawn were filtered through a 0.45 μm membrane filter and then analyzed for drug release by RP-HPLC and UV-Visible spectrophotometric method as described in section 5.3.2.

5.3.3.8. Curve fitting and release mechanism

In order to study the drug transport mechanism from the formulations used, various models were considered to fit the experimental data using Excel based DD solver to perform and evaluate dissolution data modeling. The *in vitro* release pattern was evaluated to check the goodness of fit to the zero order release kinetics [22], first order release kinetics [23], Higuchi's square root of time equation [24], Baker-Lonsdale equation [25], Hopfenberg equation [22, 26], Hixson - Crowell's cube root of time equation [27], Weibull [28] and Korsmeyer-Peppas power law equation [29, 30]. For Korsmeyer-Peppas model, data were analysed for first 60% of the drug release. The goodness of fit was evaluated using adjusted r^2 (correlation coefficient) values. This is for the reason that r^2 will always increase as more parameters are included, whereas r^2 adjusted may decrease when over fitting has occurred.

Consequently best model is the one which is having the highest r^2 adjusted rather than highest r^2 [31]. The Akaike information criterion (AIC) and Model selection criteria (MSC) criteria were calculated as discussed in chapter 5.1.3.10.1. and 5.1.3.10.2.

Additionally, the data were also fitted into Peppas- Sahlin model to understand drug release mechanism [32, 33].

5.3.3.9. Erosion studies

Tablet erosion test was performed under the same conditions described in the dissolution studies [35]. At predetermined time intervals, individual tablets were removed during the dissolution studies and placed on aluminum foil and dried at 50^0C until a constant weight was achieved which was determined with an analytical balance. The percentage of matrix eroded was calculated from the weight loss of the tablets at each time interval using the below (Eq. 2). The mean of the three tablets for each batch was used in the data analysis.

$$\text{Percent tablet eroded} = \frac{wi - wt}{wi} \times 100 \qquad (2)$$

where *wi* is the initial tablet weight and *wt* is the weight of the dried tablets.

5.3.3.10. Characterization

5.3.3.10.1. Fourier transform infra-red (FT-IR) spectroscopy

The study was undertaken in order to examine any chemical interaction between the formulation before and after sintering. The FT-IR (Bruker, USA) spectra of the pure drug, physical mixture of drug and excipients before and after sintering were investigated using potassium bromide (KBr) pellet method. In brief procedure involved mixing of approximately 2% (w/w) of the sample with respect to the KBr (S. D. Fine Chem Ltd., Mumbai, India). The mixture of drug and dry KBr was ground into an agate mortar and was compressed into a KBr pellet under a hydraulic press at 10,000 psi. Each KBr disk was scanned 16 times at 4 mm/s at a resolution of 2 cm^{-1} over a wavenumber range of 400–4000 cm^{-1}. The characteristic peaks were recorded.

5.3.3.10.2. Differential scanning calorimetry (DSC)

DSC was used for investigating any changes in the formulation before and after thermal treatment. Pure RIF, physical mixture and thermally treated samples were crimped in a standard aluminum pan and heated from 40˚C to 300˚C at a heating rate of 10˚C/min under constant purging of dry nitrogen at 40 ml/min. DSC thermograms were obtained using an automatic thermal analyzer system (DSC-60, Shimadzu, Japan). Temperature calibration was performed using indium as the standard. An empty pan, sealed in the same way as the sample, was used as the reference.

5.3.3.10.3. Powder X-Ray diffraction (PXRD)

PXRD patterns of the pure drug, physical mixture of drug and excipients before and after sintering were investigated using powder Xray diffractometer (Panalytical, XPERTPRO software, Netherlands). An X-ray beam of 2 kW was allowed to fall over the sample. As the slide moves at an angle of theta degree, a proportional detector detects diffracted X-rays at angle of 2θ° and subsequently XRD patterns were recorded.

5.3.3.10.4 Scanning electron microscopy (SEM)

The purpose of the SEM study was to obtain a topographical characterization of tablets before and after sintering as well to study effects of pore formers on tablet surface during dissolution. The microstructures of tablet surfaces might reflect the pathway for drug release. Hence, SEM study was carried out on surface of the tablets before sintering, after sintering and after 1 hour of drug release. SEM photographs were taken using a scanning electron microscope (JSM-5610LV, Jeol Ltd., Tokyo, Japan) at the required magnification at room temperature. The acceleration voltage used was 15 kV, with the secondary electron image as the detector.

5.3.3.11. Capability analysis

Capability analysis is used to assess whether a process is capable of producing output that meets your desired quality traits. A capable process is able to produce products that meet desired specifications. The process here was assumed to be in statistical control. The normal probability plot was used to examine normal distribution of data [36]. Anderson-Darling, Ryan-Joiner and Kolmogorov-Smirnov test statistic at 5%

significance level were applied to assess whether data follows a normal distribution or not. Capability analysis was performed on five reproducibility batches (n=30) using Minitab software (ver. 16.2.1., Minitab Inc., USA).Cp, CPU, CPL and Cpk were computed for potential within capability and Pp, PPU, PPL and Ppk for overall capability respectively [37, 38]. The 3-σ standard deviation variation was everywhere considered for relating process spread to specification spread.

5.3.3. 12. Packaging and stability study

The optimized batch was subjected to short term stability testing according to the ICH guidelines [39]. Tablets were packed in count of 30 into High Density Polyethylene Bottle with child resistant cap and were further induction sealed. Before induction sealed one silica bag was kept in bottle as desiccant. It was kept as for accelerated (40±2ºC/75±5% relative humidity) and long term (25±2ºC/60±5% relative humidity) stability. The samples were withdrawn periodically (0, 15, 30, 60 and 90 days) and evaluated for different physicochemical parameters like visual inspection, drug content, hardness, floating lag time and *in vitro* drug release was performed.

5.3.4. Results and discussion

5.3.4.1. QTPP of RIF oral gastroretentive dosage form

As discussed above, QTPP describes the design criteria that the drug product should possess in order to reproducibly deliver the therapeutic benefit in aspects of clinical safety and efficacy. The QTPP should be performance based and not mechanism based. Defining QTPP varies upon the type of formulation and process chosen [16, 40]. The parameters that will be focused in our study were chosen and enlisted as QTPP for RIF gastroretentive tablet. Thus, other than describing our QTPP, the steps to define the QTPP are not discussed. Though, working with the other type of formulation and its design, the importance of these steps should not be over emphasized, as they guide all the important decisions in the product development process. QTPP for RIF gastroretentive tablet is highlighted in Table 3. The depicted QTPP will lay down the basis for determining CQA.

Table 3: QTPP of RIF gastroretentive floating tablet

QTPP element	Target	Justification
Dosage form	Sustained release floating tablet	Tablet because commonly accepted unit solid oral dosage form. Floating because RIF is more absorbed from stomach due to its greater solubility and preferable site of absorption. To prevent isoniazid dependant RIF degradation by segregating its zone of delivery.
Route of administration	Oral	Dosage form designed to administer orally.
Dosage strength	150 mg	Commonly accepted strength in fixed dose combination with isoniazid and based on literature survey
Stability	Short term stability of 3 months on accelerated condition 40°C/75%RH and 3 months long term conditions 25°C/60%RH.	Minimum time period (at least 3 months initially) decided to study stability of final formulation.
Drug Product Quality Attributes	Physical Attributes	No physical defects like chipping, lamination, capping, etc.
	Assay	Meeting the compendial or other applicable quality standards (90 to 110% of label claims).
	Content Uniformity	Meeting the compendial or other applicable quality standards (90 to 110% of label claims).
	Floating lag time	Keeping as minimum as possible
	Dissolution	Initial burst release sufficient to achieve Minimum Inhibitory Concentration (MIC) followed by sustained release upto 8 hr.

Container closure system	Suitable for storage of dosage form	To maintain product integrity and quality upto target shelf life
Alternative methods of administration	None	Route of administration selected based on dosage form designed and targeted.

5.3.4.2. Risk assessment approach for deciding CQA

Based on QTPP, CQA were identified. An overall risk assessment of the drug product formulation components was performed to determine which formulation components have a high risk of impacting the drug product attributes [16, 20]. Table 4 describes risk assessment of RIF gastroretentive tablet with their respective justifications. From the Table 4 it can be revealed that hardness, assay, dissolution and floating lag time were identified as CQAs. The impact of formulation variables and unit operations on drug product quality attribute was performed using risk based matrix analysis and is depicted in Table 5 and 6 respectively. Further quantitative risk analysis was carried out using FMEA method to select the most critical factors which needed further investigation.

Table 4: Risk assessment of RIF gastroretentive floating tablet

Quality attribute of the drug products		Target	Is it a CQA?	Justification
Physical Attributes	Appearance	No visual physical defects observed in tablets.	No	Color, shape and appearance are generally set to ensure patient acceptability but since RIF is of orange-red color final color of tablet will be same. But as these are not directly linked to safety and efficacy; they are not critical.
	Odor	No unpleasant odor	No	Odor is similarly linked to patient acceptability and not directly linked to safety and efficacy. Moreover neither the drug product nor the excipients used in this product have unpleasant odor. Hence, this is not critical.
	Friability	Not more than 1.0%w/w	No	A target of NMT 1.0% is set according to the compendial requirement As friability will not impact patient safety or efficacy, this not critical.

	Hardness	Depending upon drug release profile and wax characteristics	Yes	Hardness affects drug release profile and ultimately its variability may affect product safety and efficacy. Therefore it is critical.
Assay		90% -110% of the label claim	Yes	Changes in the formulation or process variables will affect variability. Variability in assay will affect safety and efficacy; therefore, assay is critical.
Content Uniformity		90% -110% of the label claim	No	Variability in content uniformity will affect safety and efficacy. But as the drug dose is high 150 mg and will have major percentage of tablet weight (greater than 50%), there are less likely chances of variation in content uniformity. Therefore it is not critical.
Dissolution		Biphasic release: Initial burst release within first hour and thereafter sustained release for 8 hr.	Yes	Both formulation and process variables can greatly impact the dissolution profile. Thus it will be observed throughout the formulation development and optimization. Moreover failure to meet this specification will have direct impact on bioavailability and bioequivalence. Hence, it is critical.
Floating lag time		Minimum as possible	Yes	Dosage form is designed to ascertain its floating ability to get better absorbed from its preferable site of absorption in sustain manner. The lesser the floating time the lesser are the chances of its variability due to gastric emptying rate.

Table 5: Initial risk based matrix analysis for identification of impact of formulation ingredients on drug product attributes.

DP CQAs*	Wax	Pore former	Sodium bicarbonate	Filler	Aerosil 200	Magnesium stearate
Hardness	High	Low	Low	Medium	Low	Low
Assay	Low	Low	Low	Low	Low	Low
Dissolution	High	High	Low	Low	Low	Low
Floating lag time	Medium	Low	High	Low	Low	Low

DP CQAs*- Drug product critical quality attributes

Table 6: Initial risk based matrix analysis for identification of impact of unit operations on drug product attributes.

DP CQAs*	Sizing	Blending	Compression	Thermal sintering
Hardness	Low	Low	Medium	Medium
Assay	Low	Low	Low	Low
Dissolution	Low	Low	Medium	Medium
Floating lag time	Low	Low	Low	Low

DP CQAs*- Drug product critical quality attributes

5.3.4.3. FMEA approach for risk analysis

The FMEA method was used to perform the quantitative risk assessment, which could identify the modes that have the major impact on product failure, i.e., not meeting the QTPP. It is mainly helpful in assessing a new process prior to implementation which depends on product and process understanding. Here it was used to describe the effects or consequences of specific failure modes related to respective formulation variable or process parameter. The modes of failure were prioritized for risk management purposes based on the how frequently they occur, seriousness of their effects and how easily they can be detected. Those attributes that could have a high impact on the drug product attributes i.e. with high RPN, warranted further investigation whereas those attributes that had low impact on the drug product attributes required no further investigation. In the present study, the RPN ≥40 was considered as high risk, ≥20 to <40 was considered as medium risk and <20 was considered as low risk [41]. Table 7 enlists the factors that were considered in development of RIF gastroretentive tablet while performing FMEA. From the Table 7, it can be concluded that amount of wax, amount of pore former and amount of sodium bicarbonate have RPN greater than 40 and thus needed thorough investigation. Hence, their optimization was done using response surface design for establishing design space. Hardness and thermal sintering were posted moderate risk. As discussed in section 5.3.3.4., thermal sintering time was kept constant for all the batches. Hence, risk and criticality of this failure mode is low. Packaging RPN value also falls under moderate risk category. Thus stability of the final optimized formulation was undertaken to evaluate risk of this factor and is discussed in section 5.3.4.13.

Table 7: Risk assesment by FMEA analysis to identify criticality of failure modes.

Formulation/process parameter component	Failure Mode	Failure effects	S	Potential causes or root of failure	O	Detectability method or control	D	RPN
Hardness	Inadequate hardness and its range	drug release and friability	5	Machine failure, operator's error, excipient selection	3	Hardness tester, friability testing, dissolution	2	30
Amount of wax	Improper concentration	Drug release	5	Improper concentration	5	Dissolution	2	50
Amount of pore former	Improper concentration	Initial burst release	5	Improper concentration	5	Dissolution	2	50
Amount of gas generating agent (sodium bicarbonate)	Improper concentration	Floating lag time and floating duration	5	Improper concentration	5	Floating lag time and floating duration	2	50
Thermal sintering	Inadequate time	Drug release	5	Inadequate time	2	Dissolution, hardness	3	30
Packaging	Insufficient to protect drug from temperature, humidity and shipping.	Stability	5	Packaging material	3	Assay, dissolution, hardness	2	30

5.3.4.4. Preliminary trials for evaluation of type and concentration of wax

Table 8: Results of preliminary trials for evaluation of type and concentration of wax.

		MDC1	MDC 2	MDC 3	MDC 4	MDC 5	MDC 6	MDC 7	MDC 8
Sr. No.	**Ingredients**	**mg/tablet**							
1	Rifampicin	150	150	150	150	150	150	150	150
	Wax								
2	Stearic acid	30	40						
3	Glyceryl Behenate			30	40				
4	Carnabua wax					30	40		
5	Glyceryl Monostearate							30	40
	Gas generating agent								
6	Sodium bicarbonate	15	15	15	15	15	15	15	15
	Filler								
7	Microcrystalline PH 102(Avicel PH 102)	41.5	31.5	41.5	31.5	36.5	26.5	36.5	26.5
	Glidant								
8	Colloidal silicon dioxide (Aerosil P 200)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
	Lubricant								
9	Magnesium stearate	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
	Total	240	240	240	240	235	235	235	235
	Physical Evaluation								
	Hardness (Kp)-Before Curing (n=6)	2.2 - 3.3	2.3 - 3.5	2.1 - 3.4	2.3 - 3.5	2 - 3.4	2 - 3.6	2 - 3.1	2 - 2.8
	Hardness (Kp)-After Curing (n=6)	4.1 - 5.3	5.1 -6.6	4.5 - 6.3	5.1 - 6.6	5.1 - 6.4	6.2 - 7.3	3.5- 4	4.2 - 5
	Friability after curing	0.18	0.11	0.2	0.12	0.1	Nil	0.15	0.12
	Assay (n=3)	101.6± 0.9	102.5±1.1	98.6±0.5	101.8±0.8	99.9±1.5	98.2±0.9	99.6±3.2	98.9±3.8
	Weight Variation % SD (n=20)	±2.6	±3.2	±2.1	±1.9	±2.3	±2.8	±4.1	±4.6
	Floating lag time (min)	7.82±0.9	7.55±0.8	7.30±0.6	6.92±0.8	7.66±0.8	7.15±0.5	7.00±1.1	-
	Remarks	Floats	Floats	Floats	Floats	Floats	Floats	Floats	-
	Floating Time (hr)	2hr 50 min	> 4	> 4	> 4	> 4	> 4	3 hr 20 min	-
	Dissolution in 900 ml, 0.1N HCL, paddle, 50rpm								
	Time (hr)	MDC1	MDC 2	MDC 3	MDC 4	MDC 5	MDC 6	MDC 7	MDC 8
	0	0	0	0	0	0	0	0	-
	1	15.8±2.2	11.8±1.3	12.5±1.1	8±0.9	10.5±1.2	7.2±0.9	12.5±2.9	-
	2	35.6±2.1	20.7±2.9	22.8±2.8	17.8±2.6	20.8±2.3	14.5±1.8	22.8±2.8	-
	4	81.5±1.8	69.8±2.1	41.8±2.2	29.6±2.3	36.5±1.8	28.9±1.3	81.1±2.1	-

Initially effects of four different types of wax viz. stearic acid, glyceryl behenate, carnauba wax and glyceryl monostearate and their two concentrations levels were studied on *in vitro* drug release for four hours, floating time and floating lag time. The physical properties were also evaluated. The results are displayed in Table 8. Assay of all the batches were found to be good. Weight variation of all the batches showed low standard deviation except for the batches with glyceryl monostearate which showed relatively larger standard deviation. All the batches showed the hardness in the range of 2 – 3.6 Kp. This was the maximum hardness within operating range of machine. The low hardness may be due to presence of low concentration of compressible excipients like MCC PH 102 in the formulation. Friability of all the batches was below 1%.

Batch MDC 7 and MDC 8 containing glyceryl monostearate displayed poor flow and blend sticked on the turret while compression. Sticking was also observed on punches. The tablet also showed capping tendency. Though assay data are comparative good which may be due to high content of drug in it, flow issue was found and hence, only one batch was undergone for dissolution. The poor flow properties of blend were also reflected in weight variation data (Table 8). Therefore, glyceryl monosearate was dropped in further trials. Initially dissolution was low for 2 hours but at the end of 4 hours dissolution increased rapidly due to bursting of the tablet. The data are in agreement with floating time as the average floating time shown by tablets was 3 hours 20 minutes only. Here, in later hours of dissolution more media ingressed into tablet which ultimately loosen the matrix of tablet and showed faster drug release.

Batch MDC1 containing 30 mg stearic acid showed good initial release for two hours but faster release was seen in 4 hours suggesting incapable for sustaining release upto 8 hours. This may be due to low concentration of wax or after two hours hydrophobicity will be lesser and it would allow more media to ingress into tablet which may have loosen the matrix and hasten the release. On increasing concentration to 40 mg in batch MDC2 slowed down overall drug release. Initial slow down was more pronounced but at the end of 4 hours dissolution release was faster suggesting the poor matrix strength for sustaining drug release upto 8 hr. In the both the batches average floating lag time was around 7.82 min in MDC1 and 7.55 min in MDC2 which may be due to low concentration of gas generating agent. Also stearic acid pH

is acidic and drug shows incompatibility under acidic condition, stearic acid was dropped and avoided for further trials.

Batch MDC 5 and MDC 6 showed lower overall release profile including initial release as comparative to other waxes. The reason may be stronger matrix formation and more hydrophobic matrix than other waxes. In the both the batches average floating lag time was around 7.66 min in MDC5 and 7.15 min in MDC6 which may be due to low concentration of gas generating agent. Both the batches showed insufficient drug release for upto 4 hours as even 50% release was not achieved in 4 hours.

Batch containing MDC 3 showed release profile which was somewhat nearer to 50% in 4 hours. On increasing wax concentration to 40 mg in batch MDC4 slowed down overall release. In both the batches floating lag time was more around 7.30 min in MDC 3 and 6.92 min in MDC 4 which may be due to low concentration of gas generating agent.

Considering overall slow release in batches MDC3 to MDC6 further trials were planned on reducing concentration of wax to 20 mg per tablet to investigate its effect on initial fast release and overall release. For this glyceryl behenate and carnauba wax (MDC 3 to MDC6) were selected based on preliminary trials data. Concentration of gas generating agent; sodium bicarbonate was also increased to 20 mg per tablet to reduce floating lag time.

Table 9: Preliminary trials undertaken by reducing amount of wax and increasing amount of gas generating substance.

		MDC 9	MDC 10
Sr. No.	**Ingredients**		
1	Rifampicin	150	150
	Wax		
2	Glyceryl Behenate	20	
3	Carnabua wax		20
	Gas generating agent		
4	Sodium bicarbonate	20	20
	Filler		
5	Microcrystalline PH 102(Avicel PH 102)	47.6	47.6

	Glidant		
6	Colloidal silicon dioxide (Aerosil P 200)	2.4	2.4
	Lubricant		
7	Magnesium stearate		
8	**Total**	240	240
	Physical Evaluation		
	Hardness (Kp)-Before Curing (n=6)	2.2- 3.3	2.5 - 3.5
	Hardness (Kp)-After Curing (n=6)	3.3- 4.6	3.8 - 5.5
	Friability after curing	0.26	0.12
	Assay (n=3)	98.6± 1.2	101.6±1.5
	Weight Variation % SD (n=20)	±1.6	±1.3
Dissolution in 900 ml 0.1N HCL, paddle, 50 rpm			
	Time in Hr		
	0	0	0
	1	22.3±1.2	20.9±1.8
	2	54.3±2.3	46.3±2.8
	4	90.3±1.8	87.3±2.6
	Floating lag time (min)	5.6±0.8	5.9±0.6
	Floating time (hr)	2 hr 45 min	3 hr 15min

As seen from the above table decreasing the wax concentration increased the overall drug release. For batch MDC 9 containing glyceryl benehate above 50% drug released was seen in two hours and around 90% in four hours. The reason may be inadequate concentration of wax to keep strength of matrix. It was expected that initial release will be faster due to low wax concentration. But after 1 hour due to weak matrix and lower hydrophobicity it allowed higher ingress of water which further reduced matrix strength and ultimately hasten the drug released due to higher matrix erosion. For batch MDC 10 similar results were obtained. Floating lag time was decreased in both the batches MDC 9 and MDC 10 which was due to increased concentration of gas generating substances.

From the above results it was decided to keep amount of wax for both glyceryl behenate and carnauba wax 30 mg/tab as it can sustained the matrix upto 4 hr which was shown by floating time and dissolution data (Table 8). Since the drug release from the matrix was low different release enhancers or hydrophilic additives were added and evaluated. It was anticipated that judicious combination of wax and pore formers will not only provide the burst effect but will also sustain the drug release

upto 8 hr. Also the amount of gas generating agent was still enhanced to study if floating lag time is further reduced or not.

The following below mentioned hydrophilic additives or release enhancers were evaluated for its effect on *in vitro* drug release:

1. Change of filler: MCC PH 102 was replaced with lactose monohydrate (Pharmatose DCL 11) which is soluble filler. Hence, it is expected that it may hasten the drug release due to its soluble nature.
2. Addition of surfactant: Sodium lauryl sulphate (SLS) was incorporated in the tablet as by including a surfactant the release rate could be further increased as surfactants adjust the hydrophobic interactions between the drug and the matrix.
3. Addition of hydrophilic material as pore former: HPMC E5 was used as pore former. It is expected that hydrophilic material will dissolve faster upon contact with dissolution medium leaving behind pores to allow more ingress of water in the matrix and hasten the drug release.
4. Addition of channelizing agent: Sodium chloride was used as channelizing agent. It will work similar to as pore formers. It will dissolve upon contact with medium leaving behind channels for diffusion of the drug.

The results of the above study in depicted in Table 10.

Table 10: Effect of various release enhancers/hydrophilic additives on *in vitro* drug release and other physical characteristics

		MDC 11	MDC 12	MDC 13	MDC 14	MDC 15	MDC 16	MDC 17	MDC18
Sr. No.	**Ingredients**								
1	Rifampicin	150	150	150	150	150	150	150	150
	Wax								
2	Glyceryl Behenate	30	30	30	30				
3	Carnabua wax					30	30	30	30
	Gas generating agent								
4	Sodium bicarbonate	25	25	25	25	25	25	25	25
	Pore former								
5	SLS		4.6				4.6		
6	Sodium Chloride				10				10
7	HPMC 5 cps			10				10	
	Filler								
8	MCC PH 102		26.8	21.4	21.4		26.8	21.4	21.4
9	Lactose (Pharmatose DCL 11)	31.4				31.4			
	Glidant								
10	Aerosil	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
	Lubricant								
11	Magnesium stearate	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
	Total	240	240	240	240	240	240	240	240
	Physical Evaluation								
	Hardness (Kp)- Before Curing (n=6)	2.2 - 3.3	2.1 - 3.3	2.2 - 3.5	2.5 - 3.3	2.3 - 3.5	2 - 3.2	2 - 3.3	2.3 - 3.5
	Hardness (Kp)- After Curing (n=6)	4.4- 5.8	4.2 - 6.2	4.5 - 6.6	4.6- 5.8	5.5 - 6.6	5.3 - 6.3	5.5 - 6.9	5.1 - 6.2
	Friability after curing	0.15	0.1	0.12	0.18	0.11	0.12	0.12	0.20
	Assay (n=3)	102.6± 0.9	100.3±1.1	98.3±0.5	101.5±0.8	98.9±1.2	100±0.9	99.3±1.2	98.1±0.5
	Weight Variation % SD (n=20)	±2.1	±1.9	±2.8	±1.3	±2.0	±2.3	±1.5	±1.8
Dissolution in 900 ml 0.1N HCL, paddle, 50 rpm									
	Time in hr								
	1	13.1±0.9	12.9±0.8	20.3±1.1	13.9±0.8	11.3±1.5	11.1±0.8	16.5±1.3	10.9±0.9
	2	26.2±1.2	24.6±1.8	35.6±1.2	26.3±2.1	20.8±2.3	22.3±1.8	30.2±2.8	25.9±1.9
	4	73.9±2.2	46.8±2.6	58.5±2.1	46.5±2.3	66.6±2.8	40.9±2.9	50.5±2.3	44.9±2.8
	8	93.2±2.3	76.7±3.1	92.8±2.8	78.9±3.2	95.1±3.3	66.5±2.3	87.9±3.2	70.2±3.3
	Floating lag time (min)	4.10±0.5	4.12±0.8	3.90±0.8	4.22±0.9	4.50±0.6	4.80±0.6	4.54±0.8	4.69±0.6
	Floating time (hr)	6 hr 20 min	> 8	7 hr 30 min	>8	6 hr 35 min	> 8	> 8	> 8

Influence of various release enhancers/ hydrophilic additives on *in vitro* drug release and other physical characteristics:

1. Change of Filler: Batch MDC 11 and MDC 15 containing glyceryl behenate and carnauba wax respectively were compressed using lactose monohydrate (Pharmatose DCL 11) as soluble filler. The results revealed that initial release upto two hours did not increased significantly but after two hours significant increase in the drug release was observed as compare to batch containing MCC PH 102 as filler. The reason for initial low release may be due to hydrophobic surface of wax which initially doesn't allow much ingress of medium. Secondly it may be due partial coating of wax upon melting in the matrix which may result in partial coating on lactose particles and also on the drug. As time progresses more and more media will come into contact with matrix solubilizing lactose which will participate to form pores and channels within matrix. Hence, as lactose solubilizes more pores are formed and simultaneously weakens the matrix which lead to speed up the dissolution rate during 4^{th} and 8^{th} hour dissolution time points.
2. Addition of surfactant: The interaction between hydrophobic wax carrier and drug might hinder the complete release of the drug. Surfactant can balance hydro-lipid property of the release environment. So the drug concentration gradient can be bridged and maintained continuously with the existent of surfactant. Results revealed that surfactant did not hasten the drug release much compare to without surfactant. The reason may be SLS is hydrophilic surfactant and might not have better solubility in lipid matrix. The reason for slight increase in drug release might be attributed due to surfactant acting as the diffusion aid for the dissolved drug out of hydrophobic pores or channels.
3. Addition of hydrophilic substance as pore former: The batch MDC 13 showed good initial release and more than 90% drug release at the end of 8^{th} hr. While batch MDC 17 showed similar results but with slight decrease overall drug release profile.

   The reason for initially high release may be water soluble HPMC will be dissolved faster and the pores induced within short time will result in initial higher release especially in MDC 13. The dissolved HPMC will induce high porosity and

produce even more channels in the matrix, which will facilitate the penetration of the dissolution medium into the matrix and dissolve the drug more rapidly. After the prophase release drugs embedded inside the matrices could be released through the formed porous waxy networks completely.

4. Addition of the channelizing agent: Sodium chloride was added as channelizing agent. Results revealed that it did not exhibit much effect on initial drug release but hasten the drug release in later hours of dissolution. In neither batch MDC14 and MDC18 complete drug release was obtained suggesting it was inefficient here. The reason may be longer time taken by it to form the channels and formed channels may be too narrow for faster diffusion of the drug as compare to pores formed by HPMC. In later hours of dissolution sufficient channels are formed and matrix also loosens its strength which resulted in somewhat faster drug release in later hours of dissolution.

RIF is an anti-microbial agent and its sufficient amount should be released initially as loading dose to achieve its Minimum Inhibitory Concentration (MIC) to elicit required therapeutic effect in the body. On basis of its MIC, volume of distribution and fraction bioavailable, a minimum of 17.11 % should be released as initial loading dose theoretically. Considering this criteria and preliminary trials taken above, batch MDC 13 was selected for further optimization as it not only showed satisfactory initially release but also exhibited more than 90% drug release at the end of 8^{th} hour of dissolution. Moreover, amount of wax, pore former and gas generating agent were under high risk category according to FMEA analysis and hence their optimization was systematically done using design of experiment employing Box-Behnken experimental design for optimization.

5.3.4.5. Box-Behnken experimental design (BBD)

A BBD with 3 factors, 3 levels, and 15 runs was selected for the optimization study [42, 43]. Independent and dependent variables with their constraints are listed in Table 11. Q1, Q4, Q8 and floating lag time were selected as dependant variables while floating duration will be observed for each Design of Experiments (DOE) batch and will be correlated with other dependant variables. For predicting the optimal region, the quadratic equation generated for the variables was explained as follows (Eq. 3):

$$Y = \beta_0 + \sum \beta ixi + \sum \beta ijxixj + \sum \beta iixi^2 \quad (3)$$

where Y is the predicted response, β_0 is model constant/ coefficient, βi is the linear regression coefficient, βii is the squared regression coefficient, βij is the interaction effect regression coefficient and Xi is the dimensionless coded value of the independent variables (Xi). All statistical treatments of DOE were performed using Design Expert software (ver. 8.0.7.1., Stat- Ease Inc., USA) Main effect plots, interaction plots, residual plots and overlaid contour plots were generated using Minitab software (ver. 16.2.1., Minitab Inc., USA). All experimental trials were randomized to exclude any bias. Further the model was evaluated for best fit using parameters, coefficient of determination (r^2), adjusted r^2 (Adj r^2), predicted r^2 (Pred r^2), adequate precision [44] and Q^2 [45].

Table 11: Formulation variables and their levels for Box-Behnken design.

Factors	Coded levels	Actual Levels
X1: Amount of sodium bicarbonate (mg/tab)	-1	15
	0	20
	1	25
X2:Amount of HPMC (mg/tab)	-1	5
	0	10
	1	15
X3:Amount of glyceryl behenate (mg/tab)	-1	25
	0	30
	1	35
Responses	Constraints	
Q1:Percent drug released in 1 hr	18%≤Q1≤25%	
Q4:Percent drug released in 4 hr	50%≤Q4≤65%	
Q8:Percent drug released in 8 hr	85%≤Q8≤100%	
Floating lag time (min)	As minimum as possible	
Floating duration (hr)	≥ 8 hr	

5.3.4.6. Physical evaluation of tablets

Physical appearance, hardness, friability and assay of all the formulations of DOE batches were found to be satisfactory. Hardness was found to be 2-3.5 Kp for unsintered tablets which increased after thermal treatment to 4.5 - 6.8 Kp. The increase in temperature boosted up the hardness which seems to be due to the fusion of the wax particles or the formation of welded bonds among the matrix particles after cooling [46]. The thickness of all the tablets was found in range of 5.3 to 5.5 mm. The hardness of all the batches was in comparable range and statistically no major difference in its range was found amongst different batches. Hence, the risk and

criticality of this failure mode is low. Secondly as discussed in section 5.3.3.4., thermal sintering time was kept constant for all the batches. Hence, risk and criticality of this failure mode is low. Friability was found to be less than 0.5% (w/w). The formulated tablets showed less weight variation and complied with the weight variation and assay tests according to USP Pharmacopoeial limits.

5.3.4.7. Effect of factors on the responses

5.3.4.7.1. Q1: Percent drug released in 1 hr

Results of the measured response for Box-Behnken design is depicted in Table 12. ANOVA results and regression coefficients of response variables are shown in Table 13. The fitted polynomial equations relating the response to the transformed factors are shown in Table 14. Values of "Prob > F" less than 0.05 indicate model terms were considered significant and the terms having Prob > F value over 0.05 were omitted in the reduced model [44]. From the results it can be concluded that amount of HPMC was the most influencing factor affecting initial burst release to achieve desired MIC. It affected positively as of positive coefficient; Table 13; i.e. response increases with increase in factor level. The same can be inferred from the contour plots for Q1 (Fig. 4). The main effect plots Fig. 5A; were also in agreement depicting as the amount of HPMC increased from -1 level to 1, the initial percentage released increased simultaneously. However, amount of glyceryl behenate was affecting negatively (negative coefficient; Table 13; i.e. response decreases with increase in factor level) in significant amount. The main effects plots also describes the same as the amount of glyceryl behenate increased from -1 to 1 level percentage initial released decreased. High values of the square root correlation coefficient (*r2*) for all dependent variables indicate a good fit. Adj r^2 and Pred r^2 values were in reasonable agreement particularly for both full and reduced model, signifying good model fit for model. The Adeq precision measures the signal to noise ratio and value greater than 4 is desirable [44]. The values found for full and reduced model were 29.008 and 39.548 indicating an adequate signal. Q^2 value for both full and reduced model indicated good predictive power of the model (Table 14). The reason for initial higher release with HPMC is because HPMC will be dissolved faster and the pores induced within short time resulted in initial higher release. The dissolved HPMC will induce higher porosity and produce even more channels in the matrix, which will facilitate the

penetration of the dissolution medium into the matrix and/or more matrix erosion and dissolve the drug more rapidly. Solubilisation of HPMC in the matrix imparted channeling effect in matrix providing extended pathways for the diffusion of dissolution fluid as well as for dissolved drug. Similar mechanisms of influence of hydrophilic materials in waxy carrier on drug release rate have been reported by Feng-Qian Li et al [47]. Imaging technique of SEM can offer useful information about the surface characterization of the tablet surface. The microstructures of tablet sections might reflect the pathway for drug release. So, SEM was used to image the tablet surfaces before and after sintering and after 1 hr of the drug released. SEM micrographs (Fig. 6B) of the surface of the tablets after sintering show that a smooth structure covering the entire surface, promoting a better distribution of glyceryl behenate throughout the matrix and increasing the bonding strength. Thus the heat treatment causes the wax to melt, redistribute and coat drug and excipient particles, thereby creating new surfaces with lower wettability. The melting and resolidification of the polymer, due to the thermal treatment have apparently resulted in a redistribution of the polymer throughout the matrix and a possible change in nature of the pores within the matrix with decreasing the porosity and increasing the tortuosity factor of the matrix which is responsible for the retardation of drug release from matrix tablets with increasing glyceryl behenate amount in tablet. Similar mechanisms for thermal sintering of wax and its effects on drug release have been proposed by [46, 48, 49]. The SEM image of tablet surface after 1 hr (Fig. 6C) describes the porous surface which is due to HPMC dissolved as explained earlier.

The quadratic equation for full model in coded units is as below:

$$Q1 = 20.33 + 0.64\,X1 + 3.4X2 - 6.09X3 - 0.15X1X2 - 1.2X2X3 + 0.025X1X3 - 0.30X1^2 - 0.08X2^2 - 1.8X3^2 \quad (4)$$

The quadratic equation for reduced model in coded units is as below:

$$Q1 = 20.33 + 3.4X2 - 6.09X3 - 1.2X2X3 - 1.8X3^2 \quad (5)$$

Table 12: Matrix of the experiments for Box-Behnken design and results for the measured responses.

ES[a]	Amount of sodium bicarbonate (mg/tab)	Amount of HPMC (mg/tab)	Amount of glyceryl behenate (mg/tab)	Q1 (hr)[b]	Q4 (hr)[b]	Q8 (hr)[b]	Floating lag time (min)[c]	Floating time (hr)
6	-1	-1	0	15.5±1.1	46.8±2.3	85.9±3.9	7.0±0.9	> 8
9	1	-1	0	16.6±0.9	50.6±3.1	86.5±2.1	3.9±0.5	> 8
4	-1	1	0	23.6±1.6	68.8±3.9	94.8±2.1	6.9±0.3	> 7
8	1	1	0	24.1±1.3	74.6±2.2	93.9±2.0	3.8±0.3	> 7
12	-1	0	-1	23.6±0.8	79.8±3.2	92.3±3.0	7.3±0.8	> 6
1	1	0	-1	25.3±1.5	82.6±2.0	94.6±2.2	4.6±0.2	> 6
14	-1	0	1	11.1±0.9	40.8±2.5	64.0±2.0	6.7±0.3	> 8
7	1	0	1	12.9±1.6	42.8±2.0	68.0±2.5	3.5±0.5	> 8
2	0	-1	-1	20.3±1.2	83.6±3.5	94.3±1.8	5.5±0.8	> 6
13	0	1	-1	28.5±1.5	90.8±1.5	91.5±1.5	5.5±0.6	> 4
10	0	-1	1	10.8±0.8	35.6±2.3	62.0±2.1	4.1±0.3	> 8
15	0	1	1	14.2±1.5	48.6±1.5	89.5±1.2	4.2±0.2	>7
5	0	0	0	20.3±1.1	58.5±1.5	93.8±1.1	4.1±0.6	> 7
11	0	0	0	19.8±0.9	57.9±1.9	92.9±1.0	4.0±0.5	> 7
3	0	0	0	20.9±1.2	59.1±2.2	94.8±1.1	4.0±0.6	> 7

a Experimental sequence

b Mean ± SD (n = 6)

c Mean ± SD (n = 3)

Table 13: Regression analysis results.

Factors	Q1		Q4		Q8		Floating lag time	
	Coefficient	*p* value (Prob>F)	Coefficient	*p* value (Prob>F)	Coefficient	*p* value (Prob>F)	Coefficient	*p* value (Prob>F)
Intercept	20.33	< 0.0001*	58.50	0.0015*	93.83	0.0041*	4.03	< 0.0001*
X1	0.64	0.0742	1.80	0.3130	0.75	0.5930	-1.51	< 0.0001*
X2	3.40	< 0.0001*	8.27	0.0036*	5.12	0.0114*	-0.013	0.8484
X3	-6.09	< 0.0001*	-21.13	< 0.0001*	-11.15	0.0004*	-0.55	0.0003*
X1X2	-0.15	0.7234	0.50	0.8344	-0.37	0.8481	0.000	1.0000
X1X3	0.025	0.9527	-0.20	0.9332	0.43	0.8282	-0.13	0.2138
X2X3	-1.20	0.0302*	1.45	0.5511	7.57	0.0096*	0.025	0.7873
$X1^2$	-0.30	0.4984	-0.73	0.7713	-4.08	0.0888	1.03	< 0.0001*
$X2^2$	-0.079	0.8569	2.43	0.3518	0.52	0.7985	0.33	0.0148*
$X3^2$	-1.80	0.0075*	3.72	0.1757	-10.03	0.0035*	0.46	0.0041*

Regression coefficients are in coded value

* Statistically significant ($p < 0.05$)

Table 14: ANOVA results showing the effect of independent variables on the measured responses.

Measured response	Model	Sum of squares (SS)	DF	Mean square (MS)	F-value	(Prob> F) 100	PRESS	r^2	Adj-r^2	Pred-r^2	Adeq Precision	Q^2
Q 1	FM	410.18	9	45.58	71.01	<0.0001	43.01	0.9922	0.9783	0.8960	29.008	0.89
	RM	406.49	4	101.62	147.15	<0.0001	18.13	0.9833	0.9766	0.9561	39.548	0.95
Q4	FM	4226.70	9	469.63	22.79	0.0015	1638.90	0.9762	0.9334	0.6215	15.863	0.61
	RM	4117.93	2	2058.96	116.64	<0.0001	375.75	0.9511	0.9429	0.9132	31.295	0.90
Q8	FM	1860.76	9	206.75	14.96	0.0041	1081.03	0.9642	0.8997	0.4398	12.336	0.42
	RM	1790.95	4	447.74	32.23	<0.0001	520.24	0.9280	0.8992	0.7305	18.007	0.71
Floating lag time	FM	25.46	9	2.83	91.73	<0.0001	2.38	0.9940	0.9831	0.9073	28.771	0.90
	RM	25.39	5	5.08	207.33	<0.0001	0.75	0.9914	0.9866	0.9707	41.677	0.97

FM – Full Model
RM – Reduced Model

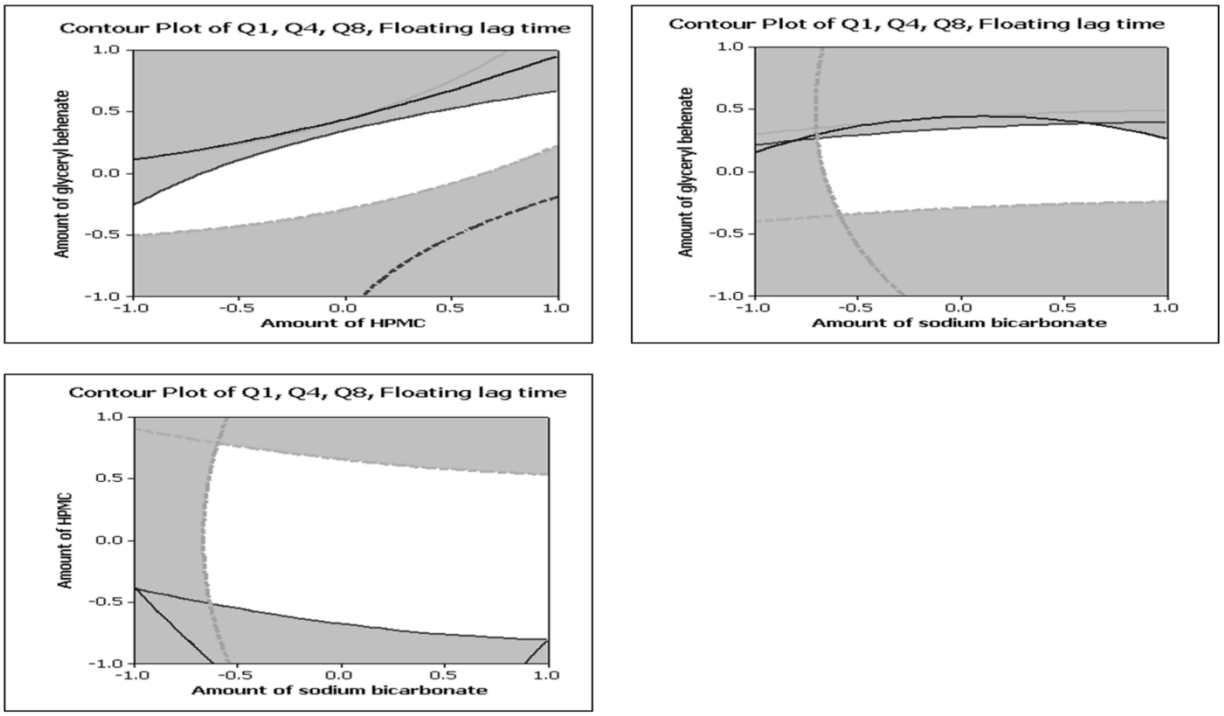


Fig.4: Overlaid contour plots of Q1, Q4, Q8 and floating lag time as a function of amount of sodium bicarbonate, amount of HPMC and amount of glyceryl behenate.

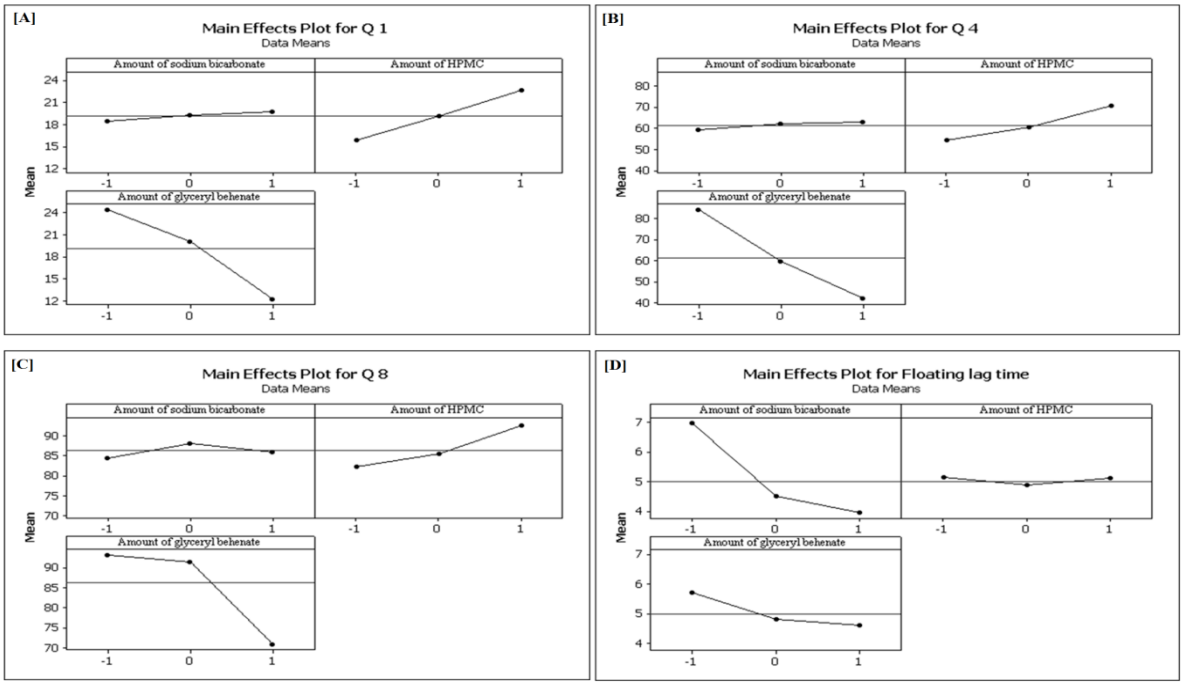


Fig.5: Main effects plot for (A) Q1, (B) Q4, (C) Q8 and (D) floating lag time as a function of amount of sodium bicarbonate, amount of HPMC and amount of glyceryl behenate.

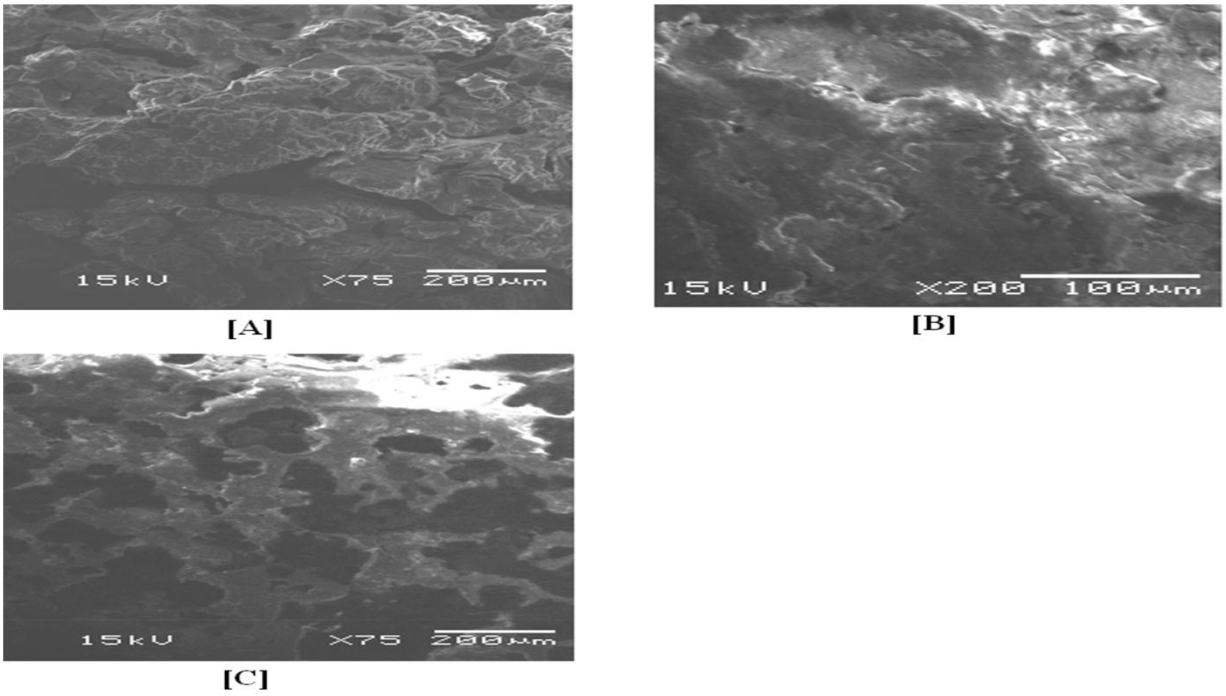


Fig.6: SEM images of tablet surface (A) without heat treatment, (B) with heat treatment and (C) after dissolution.

5.3.4.7.2. Q4: Percent drug released in 4 hr

From Table 13 and Fig. 5B, it can be concluded that amount of glyceryl behenate was the most influencing factor affecting Q4 negatively (negative co-efficient; Table 13) to maintain the sustain release. On the contrary, amount of HPMC was affecting positively; (Table 13, Fig. 5B) in noteworthy amounts. High values of the square root correlation coefficient (*r2*) for all dependent variables indicate a good fit. Adj r^2 and Pred r^2 values were in reasonable agreement for reduced model than for full model, signifying good model fit of reduced model. The Adeq precision found for full and reduced model were 15.863 and 31.295 indicating an adequate signal. Q^2 value for both full model was found to be 0.61 and for reduced model 0.90 indicating better and good predictive power of the reduced model (Table 14). The basic mechanism as explained earlier for HPMC creating positive effect and glyceryl behenate creating negative effect is that dissolved HPMC will induce high porosity and produce even more channels in the matrix, which will facilitate the penetration of the dissolution medium into the matrix and dissolve the drug more rapidly. After the prophase release drugs embedded inside the matrices could be released through the formed porous waxy networks completely. In batches (Table 12) where wax amount was at lower level (-1); experimental sequence (ES) 12,1 and pore former amount at moderate level (0) or wax amount at lower level and HPMC at lower or higher level(-1) or (1) in ES 2 and 13 respectively more than 75% drug released in 4 hour suggesting incapable for sustain release upto 8 hours. This can be correlated with floating duration as seen from Table 12. This may be due to low concentration of wax and/or high amount HPMC which will create weak matrix and low hydrophobicity penultimately allowing higher ingress of water which further reduces matrix strength and ultimately releasing higher drug due to higher matrix erosion. Even in the batches ES 8 where HPMC level is at high level (1) and glyceryl behenate at moderate level (0) more than 70% drug has been released in 4 hr. Contrary in batch ES 10 where glyceryl benehate concentration is high and HPMC at lower level release is upto 35% only in 4 hours which may be due to more hydrophobicity and less porous surface. The same can be correlated with floating duration (Table 12). Hence, there is an optimum range of glyceryl behenate and HPMC where you can get desired % release in the constraint range which is represented in both overlay contour plot (Fig. 4).

The quadratic equation for full model in coded units is as below:

$Q4=58.50+1.80X1+8.27X2-21.13X3+0.50X1X2+1.45X2X3-0.20X1X3-0.73X1^2+2.43X2^2+3.72X3^2$ (6)

The quadratic equation for reduced model in coded units is as below:

$Q4 = 58.50+8.27X2-21.13X3$ (7)

5.3.4.7.3. Q8: Percent drug released in 8 hr

As shown in Fig. 5C and Table 13 it can be concluded that amount of glyceryl behenate was the most influencing factor affecting Q 8 negatively and amount of HPMC was affecting positively in noteworthy amount. The batches (eg. ES 5, 11 and 3) in which matrix was strong enough after pores created by HPMC could sustain drug release upto 8 hr and simultaneously have released drug more than 85%. On the contrary, batches; ES 14 and 10 in which glyceryl behenate effects was prominent could sustain for 8 hr but could not release drug more than 85%. High values of the square root correlation coefficient (r^2) for all dependent variables indicate a good fit. Adj r^2 and Pred r^2 values were in reasonable agreement for reduced model than for full model, signifying good model fit of reduced model. Better Pred r^2 obtained for reduced model might be due to elimination of insignificant terms. The Adeq precision found for full and reduced model were 12.336 and 18.007 indicating an adequate signal. Q^2 value for full model was found to be 0.42 and for reduced model 0.71 indicating better and good predictive power of the reduced model (Table 14).

The quadratic equation for full model in coded units is as below:

$Q8 = 93.83+0.75X1+5.12X2-11.15X3-0.37X1X2+7.57X2X3+0.43X1X3-4.08X1^2+0.52X2^2-10.03X3^2$ (8)

The quadratic equation for reduced model in coded units is as below:

$Q\ 8 = 93.83+5.12X2-11.15X3+7.57X2X3-10.03X3^2$ (9)

5.3.4.7.4. Floating lag time

As highlighted in Fig. 5D and Table 13; amount of sodium bicarbonate was the most influencing factor affecting negatively (negative co-efficient; Table 13) floating lag time. However, surprisingly, amount of glyceryl benehate was also affecting negatively in noteworthy amount but not as significant as amount of sodium

bicarbonate. This may be due to glyceryl behenate acting as floating enhancer which may be hypothesized due to hydrophobicity provided by it. High values of the square root correlation coefficient (r^2) for all dependent variables indicate a good fit. Adj r^2 and Pred r^2 values were in reasonable agreement for both full and reduced model, signifying good model fit. The Adeq precision found for full and reduced model were 28.771 and 41.667 indicating an adequate signal. Q^2 value for both full model was found to be 0.90 and for reduced model 0.97 indicating better and good predictive power of the reduced model.

The quadratic equation for full model in coded units is as below:

Floating lag time = 4.03-1.51X1-0.013X2-0.55X3+0.0X1X2+0.025X2X3-0.13X1X3 $+1.03X1^2 +0.33X2^2+0.46X3^2$ (10)

The quadratic equation for reduced model in coded units is as below:

Floating lag time = $4.03-1.51X1-0.55X3+1.03X1^2+0.33X2^2+0.46X3^2$ (11)

5.3.4.7.5. Interaction between the factors and residual plots

An interaction is the failure of a particular factor to produce the same effect on the response at the different levels of the other factor. The ANOVA results (Table 13) and Fig. 7 depicts the interaction effects amongst the factors. Based on the *p* value X2X3 showed significant influence on percent drug release at Q1 and Q8 time point. The residual plots viz., normal probability plot of residuals, residual vs fit, residual vs order and histogram of residuals for Q1, Q4, Q8 and floating lag time are depicted in Fig. 8. The normal probability plot of residuals for responses reveals that the residuals appear to follow straight line and thus existence of non-normality, outliers, skewness or unidentified variables can be ruled out. From the plot of residual vs fit of all responses, it can be stated that residuals appear to be randomly scattered about zero and existence of missing terms, non-constant variance, outliers or influential points can be ruled out. Similar conclusions can be drawn out from histograms of residuals of all responses that skewness or outlier does not exist. Residual vs order is specifically helpful to determine whether the order of the observations influence the results or not. From the Fig.8, there exists no evidence of the error terms to be correlated with each other.

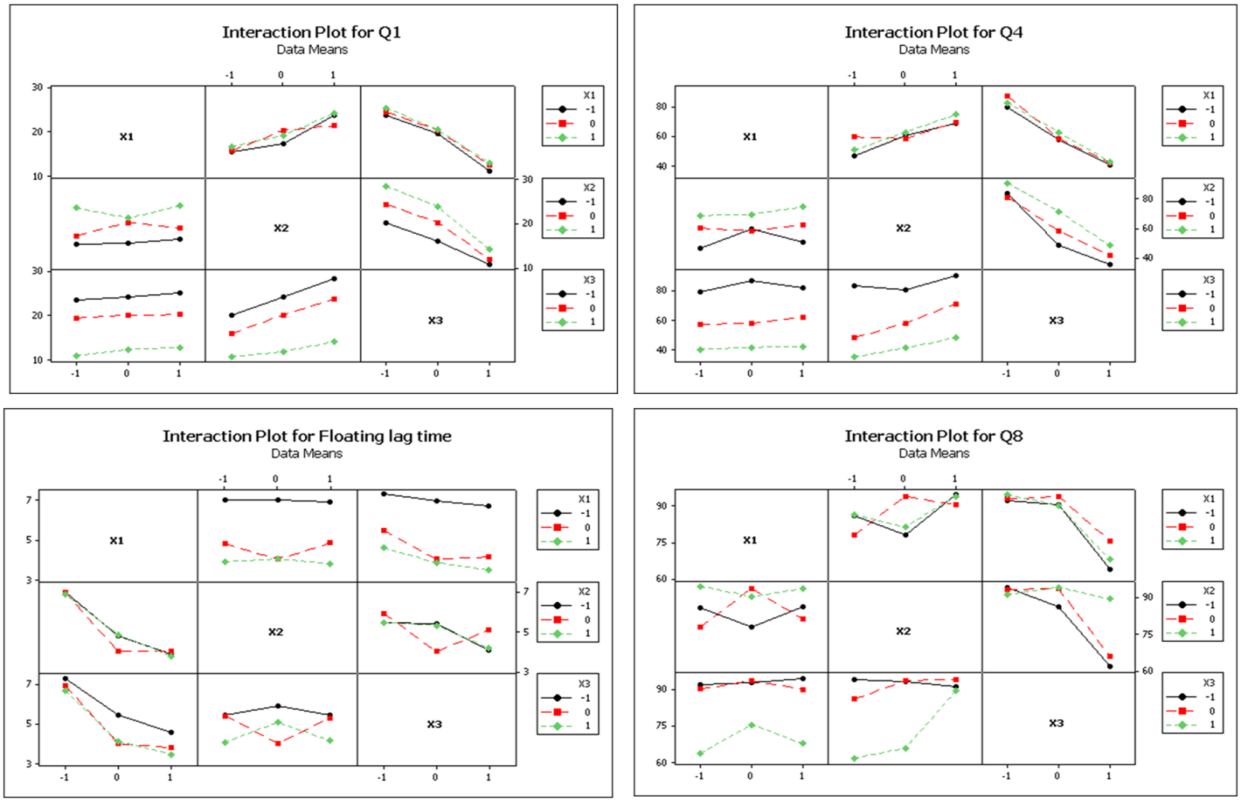


Fig.7: Interaction profile of amount of sodium bicarbonate, amount of HPMC and amount of glyceryl behenate on (A) Q1, (B) Q4, (C) Q8 and (D) Floating lag time.

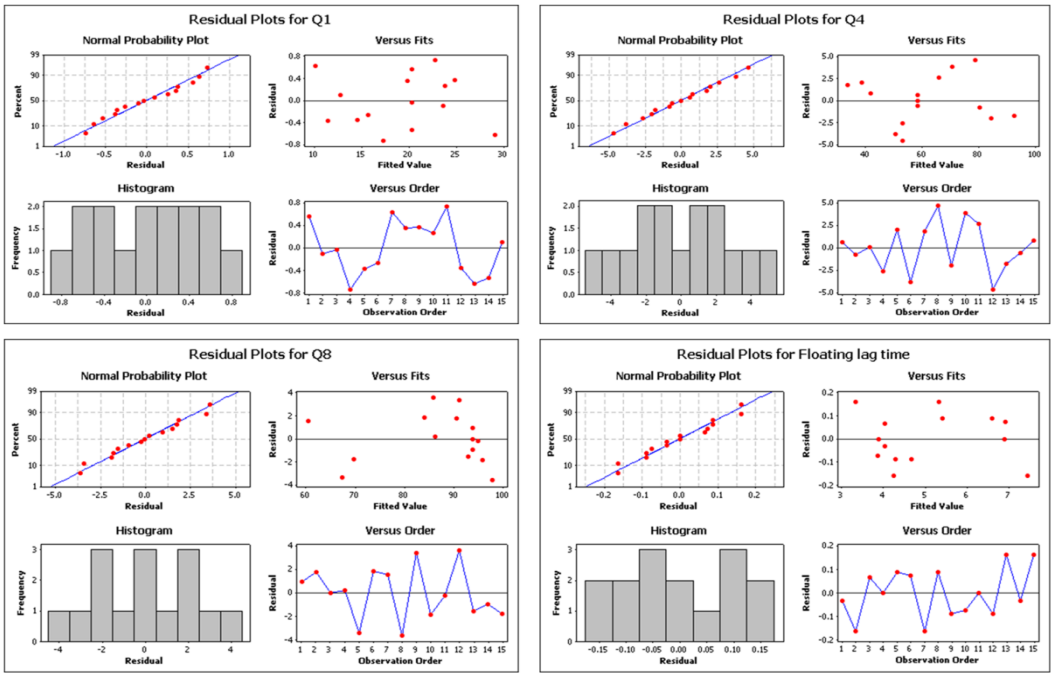


Fig.8: Residual plots for (A) Q1, (B) Q4, (C) Q8 and (D) Floating lag time.

5.3.4.7.5. Evaluation of model using cross-validation

In order to assess reliability of the model, five experiments were conducted by varying the formulation variables at values other than that of the model. The predicted and experimental values for each response are shown in Table 15. Bias or percent relative error between predicted and experimental values for each response was calculated by the following Eq. 12:

$$\text{Bias} = \left[\frac{\text{predicted value} - \text{experimental value}}{\text{predicted value}} \right] \qquad (12)$$

There was a reasonable agreement between the predicted and the experimental value in all the five batches, due to low value of the bias was found. Thus it can be concluded that the equations express adequately the influence of the selected formulation variables on the responses under study.

Table 15: Comparison of responses between predicted and experimental values for the cross validation set.

Responses	Test	Factors/levels			Experimental values	Predicted values	Bias%
		X1	X2	X3			
Q1	1	−1	-0.6	-0.6	19.10	19.82	3.63
	2	-0.6	0	0.4	17.92	17.11	-4.73
	3	-0.4	0.6	0	21.65	22.07	1.90
	4	0	-0.4	0.6	15.23	14.94	-1.94
	5	0.5	0.5	-0.5	25.90	25.11	-3.15
Q4	1	−1	-0.6	-0.6	64.85	65.56	1.08
	2	-0.6	0	0.4	50.10	49.35	-1.52
	3	-0.4	0.6	0	64.26	63.38	-1.39
	4	0	-0.4	0.6	42.96	43.89	2.12
	5	0.5	0.5	-0.5	72.30	75.27	3.95
Q8	1	−1	-0.6	-0.6	95.50	96.96	1.51
	2	-0.6	0	0.4	92.60	90.74	-2.05
	3	-0.4	0.6	0	96.12	101.23	5.05
	4	0	-0.4	0.6	86.98	84.75	-2.63
	5	0.5	0.5	-0.5	95.80	101.85	5.94
Floating lag time	1	−1	-0.6	-0.6	6.90	7.12	3.09
	2	-0.6	0	0.4	5.30	5.19	-2.12
	3	-0.4	0.6	0	5.18	4.91	-5.50
	4	0	-0.4	0.6	4.10	3.92	-4.59
	5	0.5	0.5	-0.5	4.15	4.02	-3.23

5.3.4.8. Optimization using desirability function

The desirability function was applied to merge multicriteria responses in one single criterion measurement and reveal the possibility of predicting optimum levels for the independent variables. If the value of the response is on target or is at optimum, its desirability value was assigned as 1 and for totally unacceptable value its desirability was given as 0. The individual desirability for each response was calculated [44] using the approaches discussed below.

Q1 was desired to be the maximum so as to achieve initial burst release of RIF. Desirability *d1* for response Q1 was calculated by Eq. 13:

$$d1 = \left[\frac{Yi - Ymin}{Ymax - Ymin} \right] \qquad (13)$$

Yi is the experimental result, and *Ymin* and *Ymax* represent the minimum and maximum possible values. *Ymax* and *Ymin* for this response were 28.5 and 10.8 percent drug release respectively.

For Q4 there were no specific requirements for either obtaining maximum or minimum value. Q4 response justifies that the drug releases in sustain manner from the dosage form. The formulations having percentage release within 50% to 65% were considered as optimum having desirability of 1, while formulations having values out of this range have a desirability of 0. This can be explained by below Eq. 14:

$d2 = 0$ for $Yi < Y\,min$

$d2 = 1$ for $Ymin < Yi < Ymax$

$d2 = 0$ for $Yi > Ymax$ (14)

However for Q8 more than 85% drug should be release to ascertain complete release from the dosage forms. *Ymax* and *Ymin* for this response were 94.8 and 62.0 percent drug release respectively. Thus *d3* it was calculated with same formula as Eq. 13.

Floating lag time was desired as minimum as possible. *Ymax* and *Ymin* for this response were 7.3 and 3.5 min respectively. Thus desirability function for *d 4* was calculated using the following Eq. 15.

$$d4 = \left[\frac{Ymax - Yi}{Ymax - Ymin} \right] \qquad (15)$$

The overall desirability was calculated from the individual values by using the following Eq. 16.

$D = (d1 \times d2 \times d3 \times d4)^{1/4}$ (16)

Based on the composite desirability data and overlay contour plots, ES 15 was identified as the optimum batch having desirability of 0.84. Composite desirability found out for optimized batch with the help of Minitab software was 0.86. The weight and importance was allotted 1 for each response respectively.

5.3.4.9. Curve fitting and release mechanism

Values of adjusted r^2, AIC and MSC value for various models of optimized batch ES 15 are presented in Table 16. The drug release data of the optimized batch ES 15 show a good fit to the Korsmeyer–Peppas power law release kinetics which can be confirmed by comparing the values of adjusted r^2 with that of the other models. The values of Korsmeyer–Peppas release exponent (n) determined for the optimized formulation batch ES 15 was found to be 0.706 suggesting the probable release by anomalous transport [30]. The K value of the optimized formulation was found out to be 21.974. If one considers the adjusted r^2 values, Hixon-crowell, Hopfenberg and Korsemeyer–Peppas; all the three models describe the dissolution data reasonably well. Where there are competing models (with similar r^2 values), residuals analysis can be used to distinguish between the models [31, 50]. But if there are greater number of model parameters it could lead to a higher probability of obtaining a smaller SSR value, thus AIC was applied as a substitute which renders the analysis independent of the number of parameters between models. The lowest AIC value; 8.29 of optimized batch ES 15 indicates that Korsemeyer–Peppes power low is the best fit model in describing the dissolution behavior. Another statistical tool MSC was also used to compare different models. The highest MSC; 6.54, of the optimized batch ES 15 indicates that Korsemeyer–Peppes power low is the best fit model in describing the dissolution behavior.

To investigate underlying drug release mechanism, data were fitted to Peppas-Shalin model. The value of constants k1 and k2 of Peppas –Sahlin model are displayed in Table 16. k1 denotes relative contribution of drug diffusion to drug release and k2 denotes relative contribution of polymer relaxation to drug release. From Table 16, it is clearly stipulated that erosion is the predominant mechanism for drug release. Thus the data are in agreement with the erosion studies stating polymer relaxation or erosion as predominant mechanism of drug release.

Table 16: Comparative characteristics of different drug release kinetic models for optimized batch.

Batch No:		Zero-order	First order	Higuchi	Hixon-crowell	Hopfenberg	Baker Lonsdale	Weibull	Korsmeyer Peppas	Peppas-Shalin
ES 15	r^2	0.9179	0.9728	0.9373	0.9924	0.9920	0.8474	0.9793	0.9991	0.9999
	AIC	30.49	24.97	29.14	18.56	19.38	33.59	24.16	8.29	-5.48
	MSC	2.10	3.20	2.37	4.49	4.32	1.48	3.37	6.54	9.29
									21.974 (k)	-20.50 (k1)
									0.706 (n)	41. 42 (k2)

5.3.4.10. Erosion study

The results revealed that the matrix erosion followed a linear profile with the time (Fig. 9). There was linear matrix erosion corresponding with the linear release profile of drug. Hence, study supported the fact that the release was majorly dependent upon the erosion mechanism.

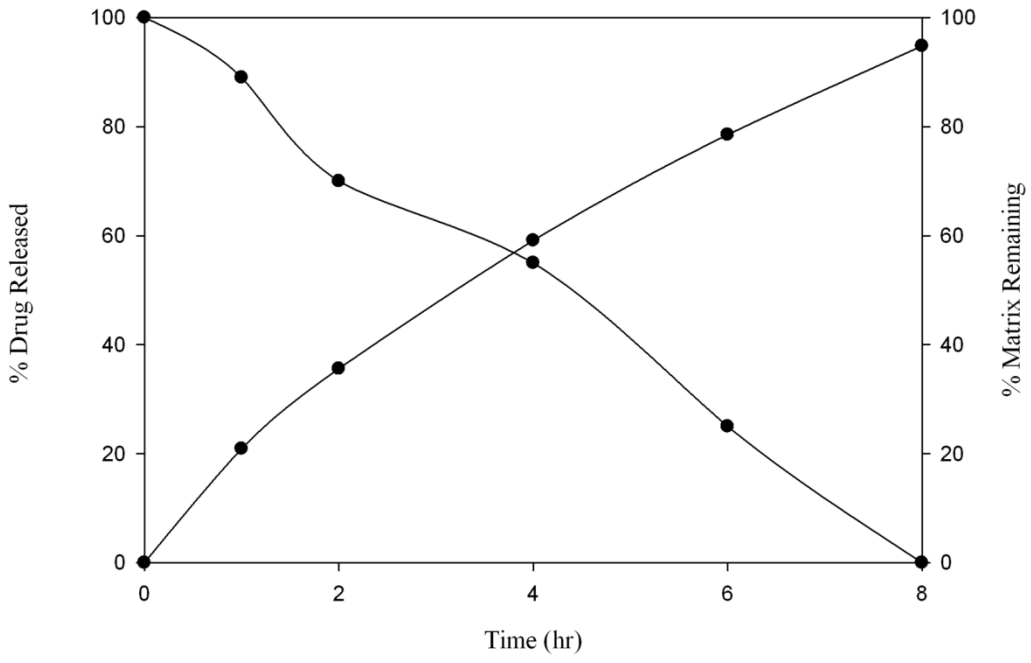


Fig.9: Comparative release and erosion profile of optimized batch.

5.3.4.11. Characterization

5.3.4.11.1. FT-IR study

During sintering there are possibilities of interaction between glyceryl benehate and RIF in which the drug release prolongation can be related to this interaction. To investigate the lack of any drug polymer interaction during sintering FT-IR spectroscopy was used. The FT-IR spectra of RIF, physical mixture of RIF and excipients and thermally treated samples were recorded and are depicted in Fig. 10. The FT-IR spectra of RIF shows characteristics of form-II signifying double peaks at 1713 and 1733 cm−1 due to acetyl and furanone C=O, respectively, broad band over 3565–3150 cm−1due to absorption of ansa OH, 1566 cm-1 due to amide C=O and 2883cm-1636 due to N-CH3 [51, 52]. This spectra was compared with physical mixture and heat treated sample. The spectra revealed no difference in the position of the absorption bands of RIF in physical mixture and heat treated samples. The spectra can be considered as the superposition of those of physical mixture and heat treated. This observation ruled out the possibility of chemical interaction and complex formation between these components by thermal treatment. DSC analysis was also used to reveal the formation of solid solution during heat treatment.

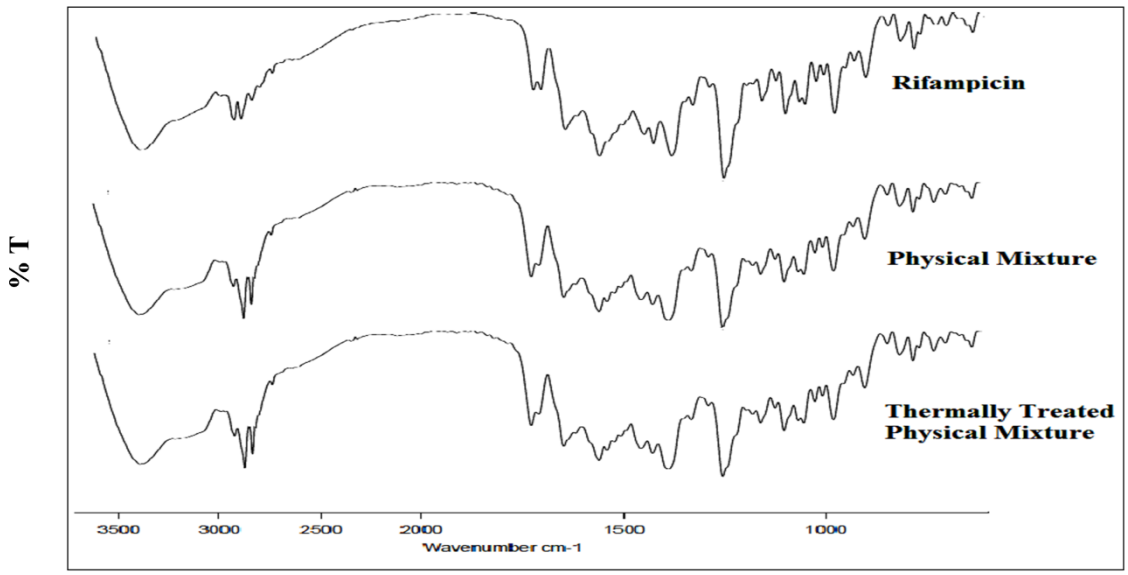


Fig.10: FT-IR spectra of plain drug, physical mixture and heat treated physical mixture.

5.3.4.11.2. DSC study

The DSC spectra of RIF, physical mixture of RIF and excipients and thermally treated samples were recorded and are showed in Fig.11. DSC spectra also revealed RIF form-II showing melting endotherm at around 186 ◦C immediately followed by recrystallization to form I (exotherm at about 196 ◦C) which is a characteristic of solid–liquid–solid transition and finally decomposes at 248 ◦C. In physical mixture, the melting endotherm at 70◦C corresponds to glyceryl behenate. Comparing the spectra of physical mixture (control) and heat treated sample shows that no change has been occurred after heat treatment, therefore there is no evidence for the formation of solid solution or polymorphic changes during the heat-treatment.

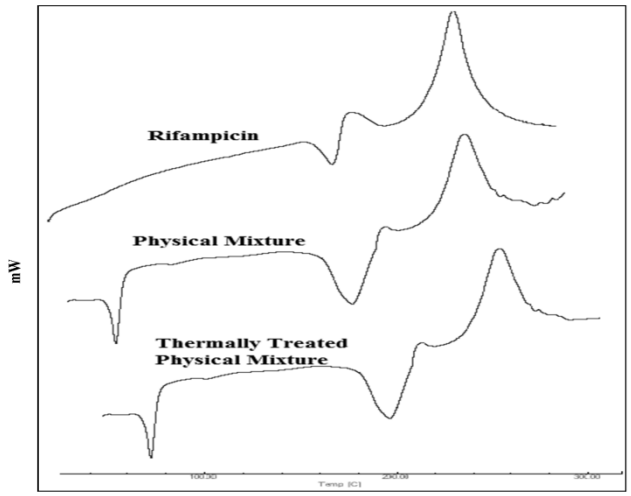


Fig. 11: DSC thermogram of plain drug, physical mixture and heat treated physical mixture.

5.3.4.11.3. Powder XRD study

Polymorphic structure of a drug is an important parameter and to investigate the effect of thermal treatment on polymorphic changes of RIF; powder XRD of RIF, physical mixture and heat treated samples were carried out. The XRD spectra of RIF (Fig. 12) occurred at 2θ at 9.91 and 11.10 which are characteristic peaks of form-II of RIF. The other characteristic were observed at 15.74 and 19.92. [51, 52]. The X ray diffraction peaks for RIF were found in the same position for both physical mixture (control) and heat-treated sample (Fig. 12). The results are is in line with the DSC and FT-IR data conforming the absence of drug polymer interaction.

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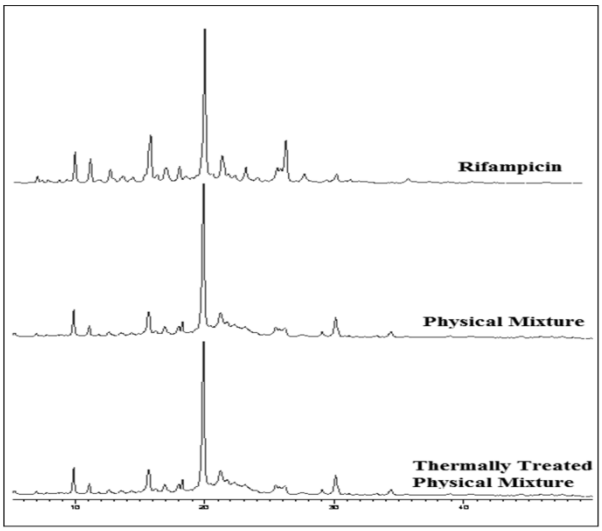

Fig. 12: PXRD spectra of plain drug, physical mixture and heat treated physical mixture

5.3.4.12. Capability analysis

The results of normal probability plots for detecting normality of distribution are depicted in Table 17 for Q1, Q4, Q8 and floating lag time respectively together with p values of Anderson-Darling test, Ryan-Joiner (similar to Shapiro-wilk test) and Kolmogorov-Smirnov test. p-values of all the three tests were greater than 0.5 indicating normal distribution of the data at 5% significance level. Hence, capability analysis with normal distribution was undertaken.

Results of the various indices of capability analysis for Q1, Q4, Q8 and floating lag time are displayed in Table 17. For a process to be capable to produce batches within specifications, all the indices value should be above 1.33 [37, 53]. From the results of Table 17, it can be revealed that all the indices value are above 1.33 which indicates that the process passes the capability analysis at 3 –σ standard deviation process spread and the process is capable of producing batches that conform to specifications. Therefore, the measurements are located within specification limits for Q1, Q4, Q8 and floating lag time.

For Q1, Cp is 2.26, which indicates that the specification spread is 2.26 times greater than the 3-σ spread in the process. Moreover Cp (2.26) and Cpk (2.21) are very close to one another, revealing that the process is centered. Also Pp (2.39), Ppk (2.33) and Cpm (2.38) are very close to another, indicating that the process is centered on the

target. Furthermore, the within and overall capability indices are very close to each other indicating process is within the control. Similar conclusions can be drawn for Q8 except for Cpm value which reveals that the process slight deviates from the target. The reason is target was set of 95% drug release which was not achieved in most cases but nevertheless all the batches showed more than 90% release assuring complete drug release at Q8. For Q4 and floating lag time, Cp and Cpk are not as close to each other as compare to Q1 signifying the process is slight deviating from the center (Table 17). Nevertheless, all the indices of within and overall were above 1.33 for Q1, Q4, Q8 and floating lag time (Table 17) and results were within desired constraints (Table 12) signifying process passes capability analysis at 3-σ standard deviation process spread. Fig. 13 portrays the pictorial representation of capability analysis for Q1, Q4, Q8 and floating lag time.

Table 17: Summary of the various capability indices

Variable	Potential within capability				Overall capability				
	Cp	Cpk	CPL	CPU	Pp	Ppk	PPL	PPU	Cpm
Q1	2.26	2.21	2.32	2.21	2.39	2.33	2.44	2.33	2.38
Q4	4.23	3.23	5.23	3.23	4.52	3.45	5.58	3.45	2.75
Q8	5.88	5.63	6.13	5.63	5.05	4.84	5.27	4.84	2.21
Floating lag time	2.67	2.17	2.17	3.16	2.83	2.30	2.30	3.35	2.26
Normal Probability test results at 5% significance level									
	AD* value	p value	RJ** value	p value	KS*** value	p value			
Q1	0.510	0.182	0.979	>0.100	0.130	>0.150			
Q4	0.520	0.171	0.982	>0.100	0.109	>0.150			
Q8	0.267	0.663	0.995	>0.100	0.093	>0.150			
Floating lag time	0.437	0.278	0.993	>0.100	0.113	>0.150			

* Anderson-Darling, ** Ryan-Joiner, *** Kolmogorov-Smirnov

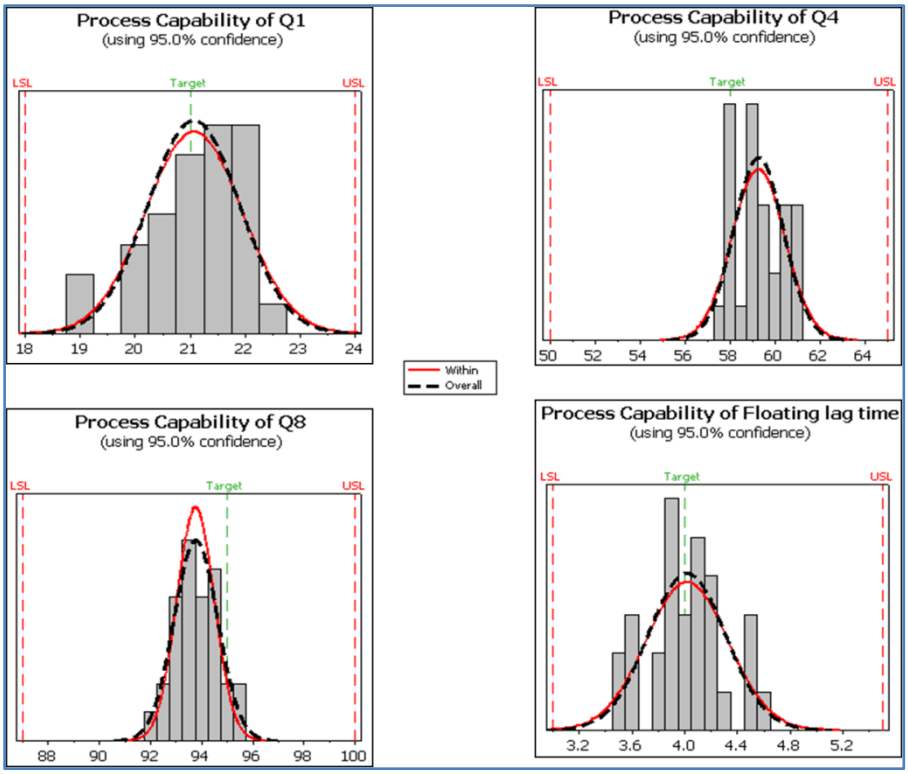

Fig. 13: Pictorial representation of capability analysis for Q1, Q4, Q8 and floating lag time.

5.3.4.13. Packaging and stability study

The optimized formulation ES 15 showed negligible change under the conditions of storage for parameters like appearance, drug content, hardness, floating lag time and *in vitro* drug release. The similarity factor (f2) [22] was employed for comparison of dissolution profiles on each time point. It ranged from 84 to 96. Thus the data suggested that the formulation was stable for under the packaging material selected revealing that it risk it under control and low.

5.3.4.14. Risk mitigation and control strategy

BBD was employed to investigate the multidimensional interaction of input variables which were ranked as high risk in the initial risk assessment for establishment of a design space. The design space is the acceptable region within which the quality of the product can be built. The broader the deign space, the more flexible and robust is your process to the changes in variations [16, 40]. The risk mitigation and control

strategy is an amalgamated outline of how quality is ascertained based on product knowledge and current process.

For X1, it can be inferred from main effect plots Fig. (5A-5D), overlay contour plots (Fig. 4) and *p* value from ANOVA (Table 13) that it significantly affected floating lag time. Thus it has minor impact on the other dependant variables (*p* value > 0.05). Main effect plots and multiple linear regression analysis results depict that the floating lag time significantly decreases from low (-1) to moderate (0) level while from moderate (0) to high (1) level it does not have major impact on decreasing floating lag time. Thus we decided to use sodium bicarbonate between 0 to 1 level which also increases its operatibility range. The risk with operating in this range is low. The risk mitigation strategy is to monitor the floating lag time.

From the ANOVA table and *p* value (Table 13), overlay contour plots (Fig. 4), interaction (Fig. 7) and main effect plots (Fig. 5A-D) it is clearly observed that both factors X2 and X3 have major impact on percent drug release at Q1, Q4 and Q8. As discussed earlier there is an optimum range and ratio of glyceryl behenate and HPMC where you can get desired % release in the constraint range which is represented in overlay contour plot (Fig. 4) of X2 and X3 vs. all four responses. Working in this range, risk is low as all the responses are in the desired constraints. The risk mitigation strategy for the same is that the all the responses Q1, Q4 and Q8 are in the constraints range.

Regarding the moderate RPN failure modes, hardness and packaging were discussed in their respective sections 5.3.4.6. and 5.3.4.13. respectively. Fig. 14 describes the FMEA analysis before and after the implementation of control strategy. RPN for all the possible failure modes were below 20 which make them fall in the low range. The final and updated risk based matrix analysis after optimization is depicted in Table 18 and Table 19 respectively. It can be clearly observed from the Table 18 and Table 19 that risk and impact of formulation variables and unit operations on drug product quality attribute falls under low category. The scalability of the design space can be evaluated in the transfer from lab to pilot and subsequent scale up batch manufacturing. Thus it may be further refined based on additional experience gained during the commercial lifecycle of the production.

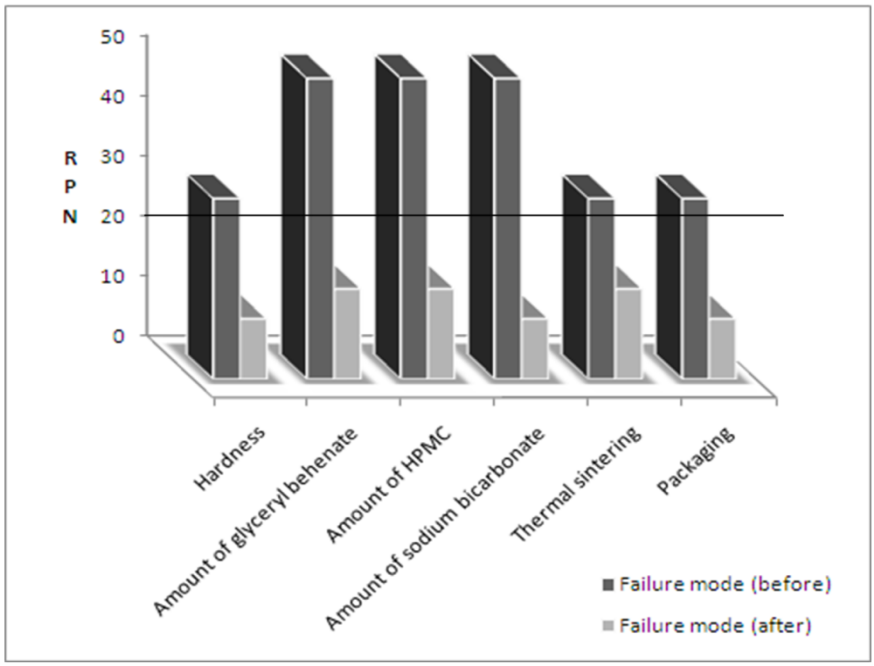

Fig.14: FMEA analysis of RIF gastroretentive tablet depicting RPN number of failure mode before and after implementation of control strategy.

Table 18: Final and updated risk based matrix analysis for identification of impact of formulation ingredients on drug product attributes.

DP CQAs*	Wax	Pore former	Sodium bicarbonate	Filler	Aerosil 200	Magnesium stearate
Hardness	Low	Low	Low	Low	Low	Low
Assay	Low	Low	Low	Low	Low	Low
Dissolution	Low	Low	Low	Low	Low	Low
Floating lag time	Low	Low	Low	Low	Low	Low

DP CQAs*- Drug product critical quality attributes

Table 19: Final and updated risk based matrix analysis for identification of impact of unit operations on drug product attributes.

DP CQAs*	Sizing	Blending	Compression	Thermal sintering
Hardness	Low	Low	Low	Low
Assay	Low	Low	Low	Low
Dissolution	Low	Low	Low	Low
Floating lag time	Low	Low	Low	Low

DP CQAs*- Drug product critical quality attributes

5.3.5. Conclusion

With the rising awareness of QbD tools and risk management approaches, the utility of it has now permeated tangibly into research and industry for understanding of process or formulation variable rationally. The research describes the overall QbD approach along with risk assessment using FMEA method, risk analysis and control strategy to mitigate the risk for development of RIF gatroretentive dosage form. In an endeavor to accomplish the objectives of QbD, response surface methodology using BBD was applied for evaluating the failure modes with high RPN number and defining the relationships between input variables and quality traits desired. The optimized formulation exhibited percent release at Q1 of 20.9%, Q4 of 59.1%, Q8 of 94.8% and floating lag time of 4.0 min. The optimization process suggests that floating lag time was majorly dependant on sodium bicarbonate and is inversely dependent upon it. The surprising findings were that it also was dependant on amount of glyceryl behenate where it was acting as floating enhancer. The percent release at Q1, Q4 and Q8 time points were directly proportional to HPMC amount and inversely proportional to amount of glyceryl behenate. The mathematical analysis of different drug release models indicated that the drug release follows Korsmeyer-peppas power law equation with anomalous transport mechanism. The AIC and MSC criteria were also in agreement stating drug release can be best described by Korsmeyer-peppas power law equation. The composite desirability for optimized formulation computed using equation and software were found to be 0.84 and 0.86 respectively. FT-IR, DSC and PXRD studies ruled out the possibilities of polymorphic transition and formation of solid solution due to thermal treatment. Finally, capability analysis was performed on reproducibility batches of optimized formulation to investigate spread of process. Anderson-Darling test, Ryan-Joiner and Kolmogorov-Smirnov test revealed normal distribution of the data. All capability indices were above 1.33 signifying process was within control of producing batches as per desired specifications. The accelerated and long term stability studies suggested negligible changes in the formulation packed in the selected packaging material. Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment depict that all the failure modes were in low risk category (Fig. 14). Thus the shift in paradigm from traditional approach to QbD approach can provide astute insight for building quality within the product.

Hence, the developed sustained release formulation may provide prudently a better substitute for immediate release tablet in circumventing its hiccups like side defects, concentration-dependent autoinduction of its own metabolism, pH dependent degradation and may anticipate a better bioavailability. The developed formulation has shown promising results *in vitro* and is potential for assessing *in vivo* bioavailability. The further *in vivo* investigations in suitable animal models and human clinical trials are required to prove the clinical usability of the experimental extended release formulation. The manufacturing method employed is relatively simple and can easily be adopted in industries.

5.3.6. References

1. Blomberg, B., Spinaci, S., Fourie, B., Laing, R., 2001. The rationale for recommending fixed dose combination tablets for treatment of tuberculosis. Bulletin of the World Health Organization. 79 (1).
2. Rifampicin-Dailymed. Available at http://dailymed.nlm.nih.gov/dailymed/drug Info.cfm? setid=6d9d13e6-246c-435c-b8c2-823eac283463
3. Rifampicin-Pubchem. Available at http://pubchem.ncbi.nlm.nih.gov/compound /6913622# section=Related-Records
4. Rifampicin-Inchem. Available at http://www.inchem.org/documents/pims/pharm/ rifam.htm.
5. Gallo, G.G., Radeilli, P., 1976. Rifampicin. In: Florey, K. (Ed.), Analytical Profiles of Drug Substances. Academic Press, New York, pp. 467–575.
6. Calvori, C., Frontali, L., Leoni, L., Tecce, G., 1965. Effect of rifamycin on protein synthesis. Nature 207, 417–8.
7. Wehrli, W., Knüsel, F., Schmid, K., Staehelin M., 1968. Interaction of rifamycin with bacterial RNA polymerase. Proc. Natl. Acad. Sci. 61, 667 73.
8. Engelberg-Kulka,H., Sat, B., Reches, M., Amitai, S., Hazan, R., 2004. Bacterial programmed cell death systems as targets for antibiotics. Trends Microbiol. 12, 66 70.
9. Campbell, E.A., Korzheva, N., Mustaev, A., Murakami, K., Nair, S., Goldfarb, A., Darst, S.A., 2001. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 104, 901–12.
10. Jack, D.B., 1992. Handbook of Clinical Pharmacokinetic data. Jack, D.B. (Ed.), Macmillian Publishers Ltd., pp. 62-63.
11. Petri, W.A., 2001. Antimicrobial Agents. In: Hardmann, J.G., Limbird, L.E., Gilman, A.G. (Eds.), The Goodman and Gilman's: The pharmacological basis of therapeutics. 10th ed., MacGraw Hill Medical Publishing division, New York, pp. 1273-1294.
12. Burman, W.J., Gallicano, K., Peloquin, C., 2001. Comparative pharmacokinetics and pharmacodynamics of the rifamycin antibacterials. Clin. Pharmacokinet. 40, 327 -41.

13. Shishoo, C.J., Shah, S.A., Rathod, I.S., Savale, S.S., Kotecha, J.S., Shah, P.B., 1999. Stability of rifampicin in dissolution medium in presence of isoniazid. Int. J. Pharm. 190, 109–123.
14. ICH harmonised tripartite guideline, November 2005. Validation of analytical procedures: text and methodology. Q2 (R1).
15. Food and Drug Administration CDER, March 2007. Draft guidance for industry and review staff: Target product profile-strategic development tool. Available at http://www.fda.gov/cder/gmp/gmp2004/GMP finalreport2004.htm.
16. Lionberger, R.A., Lee, S.L., Lee, L., Raw, A., Yu, L.X., 2008. Quality by design: concepts for ANDAs. AAPS J. 10, 268–276.
17. ICH harmonised tripartite guideline, August 2009. Pharmaceutical development Q8 (R2).
18. Fahmy, R., Kona, R., Dandu, R., Xie, W., Claycamp, G., Hoag, S.W., 2012. Quality by design I: application of failure mode effect analysis (FMEA) and Plackett–Burman design of experiments in the identification of "main factors" in the formulation and process design space for roller-compacted ciprofloxacin hydrochloride immediate-release tablets. AAPS PharmSciTech 13, 1243–1254.
19. Food and Drug Administration, 2003. Final report on pharmaceutical cGMPs for the 21st century-A risk-based approach.
20. ICH harmonised tripartite guideline, November 2005. Quality risk management Q9.
21. Badhan, A.C., Mashru, R.C., Shah, P.P., Thakkar, A.R., Dobaria, N.B., 2009. Development and evaluation of sustained release gastroretentive minimatrices for effective treatment of H. pylori infection. AAPS PharmSciTech 10, 459–467.
22. Costa P, Sousa Lobo JM (2001) Modeling and comparison of dissolution profiles. Eur J Pharm Sci 13:123–133.
23. Wagner, J.G., 1969. Interpretation of percent dissolved time plots derived from in vitro testing of conventional tablets and capsules. J. Pharm. Sci. 58, 1253–1257.
24. Higuchi, T., 1963. Mechanism of sustained action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J. Pharm. Sci 52, 1145–1149.

25. Baker, R.W., Lonsdale, H.S., 1974. Controlled release: mechanisms and rates. In: Taquary, A.C., Lacey, R.E. (Eds.), Controlled Release of Biologically Active Agents. Plenum Press, New York, pp. 15–71.
26. Katzhendler, I., Hoffman, A., Goldberger, A., Friedman, M., 1997. Modeling of drug release from erodible tablets. J. Pharm. Sci. 86, 110–115.
27. Hixson, A.W., Crowell, J.H., 1931. Dependence of reaction velocity upon surface and agitation. Ind. Eng. Chem. 23, 923–931.
28. Sathe, P.M., Tsong, Y., Shah, V.P., 1996. In vitro dissolution profile comparison: statistics and analysis, model dependent approach. Pharm. Res. 13, 1799–1803.
29. Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. Int. J. Pharm. 15, 25–35.
30. Ritger, P.L., Peppas, N.A., 1987. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J. Control. Release 5, 37–42.
31. Zhang, Y., Huo, M., Zhou, J., Zou, A., Li, W., Yao, C., Xie, S., 2010. DDSolver: an add in program for modeling and comparison of drug dissolution profiles. AAPS J. 12, 263–271.
32. Peppas, N.A., Sahlin, J.J., 1989. A simple equation for the description of solute release III. Coupling of diffusion and relaxation. Int. J. Pharm. 57,169–172.
33. Grassi, M., Grassi, G., 2005. Mathematical modelling and controlled drug delivery: matrix systems. Curr. Drug. Deliv. 2, 97–116.
34. Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans.Automat. Contr. 9, 716–723.
35. Hiremath, S.P., Saha, R.N., 2004. Design and study of rifampicin oral controlled release formulations. Drug Deliv. 11, 311–317.
36. Bissell D., 1994. Statistical Methods for SPC and TQM, Chapman and Hall, London.
37. Rudisill, F., Litteral, L.A., 2008. Capability ratios: Comparison and interpretation of short-term and overall indices. Int. J. Qual. Stand. 2(1), Paper 3, 67–86.
38. Shinde, J.H., Katikar, R.S., 2012. Importance of process capability and process performance indices in machine tool. Int. J. Res. Engin. Appl. Sci. 2, 1211-1217.

39. ICH harmonised tripartite guideline, February 2003. Stability testing of new drug substances and products Q1A(R2).
40. Yu, L.X., 2008. Pharmaceutical quality by design: product and process development, understanding, and control. Pharm. Res. 25, 781–791.
41. Hiyama, Y., 2009. Quality overall summary mock P2 March 2009. http://www.nihs.go.jp/drug/section3/English%20Mock%20QOS%20P2%20R.pdf/
42. Box, G.E.P., Behnken, D.W., 1960. Some new three level designs for the study of quantitative variables. Technometrics 2, 455–475.
43. Lewis, G.A., Mathieu, D., Phan-Tan-Luu, R., 1999. Response surface methodology, in: Pharmaceutical Experimental Design. Marcel Dekker Inc., New York, pp.185–246.
44. Shah, P.P., Mashru, R.C., Rane, Y.M., Thakkar, A., 2008. Design and optimization of mefloquine hydrochloride microparticles for bitter taste masking. AAPS PharmSciTech 9, 377–389.
45. Singh, B., Kumar, R., Ahuja, N., 2005. Optimizing drug delivery systems using systematic "design of experiments." Part I: fundamental aspects. Crit. Rev. Ther. Drug Carrier Syst. 22, 27–105.
46. Singh, R., Poddar, S.S., Chivate, A., 2007. Sintering of wax for controlling release from pellets. AAPS PharmSciTech 8, E1–E9.
47. Li, F.Q., Hu, J.H., Deng, J.X., Su, H., Xu, S., Liu, J.Y., 2006. In vitro controlled release of sodium ferulate from Compritol 888 ATO based matrix tablets. Int. J. Pharm. 324, 152–157.
48. Sudha, B.S., Sridhar, B.K., Srinatha, A., 2010. Modulation of tramadol release from a hydrophobic matrix: implications of formulations and processing variables. AAPS PharmSciTech 11, 433–440.
49. Zhang, Y.E., Schwartz, J.B., 2003. Melt granulation and heat treatment for wax matrix controlled drug release. Drug Dev. Ind. Pharm. 29, 131–138.
50. Pather, S.I., Russell, I., Syce, J.A., Neau., S.H., 1998. Sustained release theophylline tablets by direct compression. Part 1: formulation and in vitro testing. Int. J. Pharm. 164, 1-10.
51. Henwood, S.Q., de Villiers, M.M., Liebenberg, W., Lotter, A.P., 2000. Solubility and dissolution properties of generic rifampicin raw materials. Drug Dev. Ind. Pharm. 24, 403–408.

52. Agrawal, S., Ashokraj, Y., Bharatam, P.V., Pillai, O., Panchagnula, R., 2004. Solid state characterization of rifampicin samples and its biopharmaceutic relevance. Eur. J. Pharm. Sci. 22, 127–144.

53. Kane, V. E., 1986. Process capability indices. J. Qual. Technol. 18, 41-52.