# Chapter 5.2

# Quality by design enabled formulation development and optimization of enteric coated sustained release Isoniazid pellets

### 5.2.1. Analytical methods

Analytical methods as discussed in section 5.1.2. were used in the development and optimization of INH pellets.

### 5.2.2. Methods

#### 5.2.2.1. Quality Target Product Profile (QTPP) of INH site specific sustained release dosage pellets

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 [1]. 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 [2]. The International conference of harmonization (ICH) Q8 (R2) [3] 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 [4, 5].

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

Risk based compliance is an imperative FDA initiative for current Good Manufacturing Practice in the 21st century [6]. ICH Q9 [5] 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” [5]. 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.

### 5.2.2.3. Failure Mode and Effects Analysis (FMEA)

FMEA was initially developed outside of health care system and its domain is now reached in 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 [4]. 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:

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

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.

### 5.2.2.4. Drug excipient compatibility

Drug excipient compatibility was investigated using Differential scanning calorimetry (DSC) and Fourier transform infra-red (FT-IR) spectroscopy study.

### 5.2.2.4.1. DSC study

DSC was used for investigating any incompatibility between drug and excipients. Pure INH and physical mixture of drug with excipients were crimped in a standard aluminum pan and heated from 40˚C to 200˚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.

### 5.2.2.4.2. FT-IR study

The study was undertaken in order to examine any chemical interaction between the drug and excipients. The FT-IR (Bruker, USA) spectra of the pure INH and physical mixture of drug with excipients was 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.

### 5.2.2.5. Preparation of INH sustained release matrix pellets

Briefly, accurately weighed quantity of INH (75 mg/tab), microcrystalline cellulose (MCC PH 101), polymers (glyceryl benehate, carnauba wax, Eudragit RLPO, HPMC K 100 LVCR carbopol 934, Gelucire 50/3, ethyl cellulose (EC N80, N20)), binders (PVP K-30, Polyox N12K, HPC HF, HPMC K4M), were sifted through ASTM # 30 (Jayant Scientific Industries, Mumbai, India) and talc and colloidal silicon dioxide (Aerosil P 200) sifted through ASTM 60# were physically mixed for about 10 min. Finally magnesium stearate was sifted through ASTM 60# and added as lubricant and blended for 3 min. Purified water was added in sufficient quantity and the blend was kneaded to obtain coherent damp mass feasible for extrusion. Extrudates were obtain using radial screen extruder by extruding at constant speed of 100 rpm, through mesh having size of 1 mm in diameter (Cronimach, India/Umang Pharmatech Pvt. Ltd., India). A spheronizer having friction plate of 2 mm and cross hatched geometry was used for spheronization (Cronimach, India/ Umang Pharmatech Pvt. Ltd., India). The extrudates were spheronized for 3 to 6 min at 600 -1000 rpm depending upon the formulation batch processed. The pellets were dried in fluid bed processor (Pam Mini-glatt, India) at 50$^{o}$ C for 30 minutes. The dried pellets were thermally sintered at 80 $^{o}$ C for 20 minutes in hot air oven wherever wax was used (Shree Kailash Industries, India). Pellets were allowed to cool to room temperature and all the physical tests, dissolution and further processing were carried out after 24 hours of thermal treatment.

### 5.2.2.6. Seal coating on optimized core pellets

Seal coat on core pellets of optimized batch was carried out with 8% (w/w) aqueous dispersion of Hydroxy propyl methyl cellulose (HPMC E5) and polyethylene glycol (PEG 400) (82 : 18) using fluid bed processor (Pam Mini-glatt, India) to achieve 5% weight gain. The process conditions were pre-warming of the pellets at 40 $^{o}$C for 10 min; spray nozzle diameter, 0.5 mm; atomizing air pressure, 0.3-0.5 mPa; inlet air temperature, 45-55$^{o}$C; product temperature 35-40$^{o}$C; spray rate, 0.2-0.45 gm/min; air flow 0.12-0.38, post-drying at 40 $^{o}$C for 20 min. The coating was performed to achieve weight gain of 5% w/w.

### 5.2.2.7. Enteric coating on seal coated pellets

Enteric coating of pellets was done using fluid bed processor (Pam Mini-glatt, India) machine using aqueous dispersion of Eudragit L-100-55. In brief, dispersion was prepared as follows: Eudragit L-100-55 powder was slowly added into water under stirring and stirred for 5 minutes. To this 1N sodium hydroxide solution was added as stabilizer and final make up was done with water to obtain 30% dispersion. This dispersion was stirred for about 30 minutes. On the other side; triethyl citrate as plasticizer (10%w/w of dry polymer) and talc as anti-tacking agent (25% w/w of dry polymer) were homogenized in water using homogenizer (UltraTurrax, IKA, USA) for 10 minutes. Now the excipient suspension was slowly added into Eudragit L-100-55 dispersion under stirring for about 30 minutes. Final suspension was diluted with purified water under stirring to obtain 15% w/v aqueous dispersion. Finally, the suspension was passed through ASTM 60# sieve and further used. The process conditions were pre warming of the pellets at 40° C temperature for 10 minutes, spray rate (0.2-0.45 gm/min), inlet air temperature (40-50° C), bed temperature (29 - 32° C) atomizing air pressure (0.3-0.5 mPa), and air flow (0.15- 0.39 ) were adjusted. The post-drying was carried out at 40° C for upto 15 minutes. The coating was performed to achieve three different weight gains viz. 15%w/w, 20%w/w and 25%w/w.

### 5.2.2.8. Physical characterization of core matrix pellets

### 5.2.2.8.1. Usable yield

The size distribution of the matrix core pellets was evaluated by sieving method using standard set of sieves (600-1700μm). Initially, the pellets were put on the top sieve and then the set of sieves were shaken for 5 min. The fraction of pellets (600-1180μm) was considered as the usable yield [7].

### 5.2.2.8.2. Pellet size.

The average particle diameter and size distribution of microparticles were determined by using Malvern (Mastersizer 2000, Malvern Instruments, UK). An aliquot of the microparticle suspension was then added into the small volume recirculation unit and circulated at 3500 rpm. Each sample was measured in triplicate in the analysis.

### 5.2.2.8.3. Pellet shape

The images were captured using microscope and shape analysis was performed using calibrated stage microscope with optical eyepiece. Analysis was carried out using 50 pellets from usable yield fraction. In this study, aspect ratio was calculated using the following equation

$$\text{Aspect ratio} = \frac{\text{dmax}}{\text{dmin}}$$

### 5.2.2.8.4. Abrasion resistance

The abrasion resistance was evaluated using Friabilator (VFT-2D, Veego Instruments Co., Mumbai, India) by rotating 100 revolutions at 25 rpm. A fixed quantity of pellets (~10 gms) were taken from the usable fraction and placed into friabilator and rotated along with 25 steel spheres, each 2mm in diameter. After 100 revolutions the mass retained onto (600-1180μm) was gently tapped to remove fines and was reweighed. The percentage loss of mass between the initial and final weights of the pellets was taken as abrasion resistance.

### 5.2.2.8.5. Drug content

The drug content measurement was carried out by developed UV – spectrophotometric and HPLC methods as discussed in chapter 5.1.2.

### 5.2.2.9. Physical characterization of coated pellets

### 5.2.2.9.1. Drug content

The drug content measurement was carried out by developed UV – spectrophotometric and HPLC methods as discussed in chapter 5.1.2.

### 5.2.2.9.2. Weight gain

Firstly, initial weight of the fixed quantity of uncoated pellets was measured. Then the required quantity of the coating solution to achieve theoretical weight gain was sprayed. Subsequently the weight of the coated pellets was measured and the difference of weight between the coated pellets and uncoated pellets was considered as actual weight gain. For weight gain, trials were undertaken to determine process efficiency and subsequently based upon that extra coating solution was prepared.

### 5.2.2.10. *In vitro* drug release

The study was performed using United States Pharmacopeia (USP) 30 type II apparatus (VDA 6-DR, Veego Instruments Corporation, Mumbai, India) using Method B for delayed release products as specified in USP for enteric coated dosage forms [8]. Pellets equivalent to 75 mg were analyzed for dissolution profiling and data were recorded at 1, 2, 3, 4, 6, 8, and 10 h. For optimization of core matrix pellets dissolution was performed in pH 6.8 phosphate buffer. Samples withdrawn were filtered through a 0.45 µm membrane filter and then analyzed immediately for drug release. The drug released in acidic medium was analyzed as per RP-HPLC method and release in pH 6.8 phosphate buffer by UV-spectrophotometry and RP-HPLC method as described in section 5.1.2.

### 5.2.2.11. 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 [9], first order release kinetics [10], Higuchi’s square root of time equation [11], Hopfenberg equation [9, 12], Hixson - Crowell’s cube root of time equation [13] and Korsmeyer-Peppas power law equation [14, 15]. 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 $r^2$ [16]. 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. [17, 18].

### 5.2.2.12. Packaging and stability study

The optimized batch was subjected to short term stability testing according to the ICH guidelines [19]. Pellets were filled in capsule size 0 and 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. The sealed bottles were exposed to accelerated (40 ± $2^{o}$C/75 ± 5% relative humidity) and long term (25 ± $2^{o}$C/60 ± 5% relative humidity) stability for three months. The samples were withdrawn periodically (0, 15, 30, 60 and 90 days) and evaluated for different physicochemical parameters like visual inspection, drug content, gastric resistance (drug release in 0.1N HCl) and *in vitro* drug release.

## 5.2.3. Results and Discussion

### 5.2.3.1. QTPP of INH site specific sustained release pellets

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 [2, 20]. The parameters that will be focused in our study were chosen and enlisted as QTPP for INH enteric coated sustain release pellets. 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 INH site specific sustained release pellets is highlighted in Table 1. The depicted QTPP will lay down the basis for determining CQA.

**Table 1: QTPP of INH enteric coated sustained release pellets**

| QTPP element | Target | Justification |
|---|---|---|
| Dosage form | Enteric coated pellets which gives sustain action in pH 6.8 | Pellets because commonly accepted unit solid oral dosage form, uniform distribution in gastrointestinal tract, less affected gastric emptying rate and gastric transit time, less susceptible to dose dumping, attain more constant plasma levels<br>Enteric coated because to prevent INH dependant RIF degradation in acidic media by segregating its zone of delivery.<br>Secondly, INH is more absorbed from all segments of intestine due to its unionized form at intestinal pH and preferable site of absorption. |
| Route of administration | Oral | Dosage form designed to administer orally. |
| Dosage strength | 75 mg | Generally accepted strength for combination with Rifampicin (Fixed Dose Combination) |
| 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 | 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). |
| | Drug release in 0.1N HCl | Less than 10% |
| | Dissolution in pH 6.8 buffer | Initial burst release sufficient to achieve 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. - |

### 5.2.3.2. 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 [2, 5]. Table 2 describes risk assessment of INH site specific sustained release pellets with their respective justifications. From the Table 2 it can be revealed that abrasion resistance, shape, assay, dissolution in pH 6.8 buffer and gastric resistance (drug release in 0.1N HCL) 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 3 and 4 respectively. Further quantitative risk analysis was carried out using FMEA method to select the most critical factors which needed further investigation.

## Table 2: Risk assessment of INH enteric coated sustained release pellets

| Quality attribute of the drug products | | Target | Is it a CQA? | Justification |
|---|---|---|---|---|
| Physical Attributes | Appearance | No visual physical defects observed in pellets. | No | Color, and appearance are generally set to ensure patient acceptability. 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/Abrasion resistance | Not more than 0.5 %w/w | Yes | To evaluate the resistance of the pellet surface to abrasion, that pellets will encounter during further processing and shipping. Friable pellets may also create problems in coating via fluid bed processor. Moreover, friable pellets can alter drug release profile which will directly impact on patient safety and efficacy, therefore this is critical. |
| | Size | Narrow particle size distribution | No | Polydispersity is important factor to reduce variation during filling of capsule and also for uniformity of coating. Uniformity of coating affects drug release and variation during filling can affect drug content issues. Hence, it will directly impact on safety and efficacy. But here there are less chances of variation in size as pellets from usable yield will be only taken for further evaluation and processing. Hence, this is not critical. |
| | Shape | Spherical | Yes | Sphericity is required for flowability, to obtain high usable yield and uniformity in coating. As uniformity of coating is directly linked to safety and efficacy this is critical. |
| Assay | | 90% -110% of the label claim | Yes | Changes in the formulation or process variables may affect it. 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 75 mg, 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 hours. | 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. |
| Drug release in 0.1N HCl | | Less than 10% | Yes | Dosage form is designed to ascertain minimal release in 0.1N HCL to minimize interaction with RIF and to get better absorbed from its preferable site of absorption in sustain manner. Therefore it is critical. |

**Table 3: Initial risk based matrix analysis for identification of impact of formulation ingredients on drug product attributes.**

| DP CQAs* | Filler | Binder | Polymer | Aerosil P200/ Talc | Magnesium stearate |
|---|---|---|---|---|---|
| Abrasion resistance | Low | Medium | Medium | Low | Low |
| Shape | Medium | Medium | Low | | |
| Assay | Low | Low | Low | Low | Low |
| Dissolution in pH 6.8 buffer | Low | Low | High | Low | Low |
| Gastric Resistance (Drug release in 0.1 N HCL) | Low | Low | Low | Low | Low |

DP CQAs*- Drug product critical quality attributes

**Table 4: Initial risk based matrix analysis for identification of impact of unit operations on drug product attributes.**

| DP CQAs* | Sizing | Blending | Granulation | Extrusion | Spheronization | Enteric Coating |
|---|---|---|---|---|---|---|
| Abrasion resistance | Low | Low | Low | Low | Low | Low |
| Shape | Low | Low | Low | Low | High | Low |
| Assay | Low | Low | Low | Low | Low | Low |
| Dissolution pH 6.8 buffer | Low | Low | Low | Medium | Medium | Low |
| Gastric Resistance (Drug release in 0.1 N HCL) | Low | Low | Low | Low | Low | High |

DP CQAs*- Drug product critical quality attributes

### 5.2.3.3. 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 (Hiyama, 2009). Table 5 enlists the factors that were considered in development of INH site specific extended release pellets while performing FMEA. From the Table 5, it is clearly stipulated that amount of Polymer, and enteric coating have RPN ≥ 40 and require through investigation and optimization. While amount of binder, thermal sintering and packaging fall under moderate risk category.

**Table 5: Risk assessment 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 |
|---|---|---|---|---|---|---|---|---|---|
| Extrusion Process | Extruder sieve | Improper selection | Extrudability, particle size | 5 | Improper selection | 2 | Visual observation, particle size determination | 1 | 10 |
| | Extrusion speed | Improper selection | Extrudability, shape of pellets | 5 | Improper selection, machine failure, operator's error | 2 | Monitoring the process, visual observation, aspect ratio | 1 | 10 |
| | Amount of binder | Improper concentration | Usable yield, particle size distribution, shape, abrasion resistance, extrudability | 5 | Improper concentration and selection | 3 | Particle size determination, abrasion resistance, dissolution | 2 | 30 |
| | Amount of granulating liquid | Improper amount | Usable yield, shape, particle size distribution | 4 | Improper amount | 2 | Particle size determination, aspect ratio, calculation of usable yield | 2 | 16 |
| Spheronization Process | Spheronization speed | Improper selection | Shape, usable yield, coating uniformity | 3 | Improper selection | 3 | Aspect ratio, coating uniformity | 2 | 18 |
| | Spheronization time | Improper selection | Shape, coating uniformity, usable yield | 3 | Improper selection | 3 | Aspect ratio, coating uniformity | 2 | 18 |
| Formulation | Amount of Polymer | Improper concentration | Drug release | 5 | Improper concentration | 5 | Dissolution | 2 | 50 |
| | Thermal sintering (if applicable) | Inadequate time | Drug release | 5 | Inadequate time | 2 | Dissolution, hardness | 3 | 30 |
| Enteric Coating | | Improper coating | Gastric resistance | 5 | Improper weight gain, coating uniformity | 4 | Gastric resistance, dissolution | 2 | 40 |
| Packaging | | Insufficient to protect drug from temperature, humidity and shipping | Stability | 5 | Packaging material | 3 | Assay, dissolution | 2 | 30 |

### 5.2.3.4. Preliminary trials

**Table 6: Preliminary trials for selection of type of polymer**

| Sr.No. | Ingredients | Batch (%w/w) | | | | | |
|---|---|---|---|---|---|---|---|
| | | INH EX 1 | INH EX 2 | INH EX 3 | INH EX 4 | INH EX 5 | INH EX 6 |
| 1 | Isoniazid | 2 | 2 | 2 | 2 | 2 | 2 |
| 2 | MCC PH 101 | 5.85 | 5.85 | 5.85 | 5.85 | 5.85 | 5.85 |
| 3 | Glyceryl behenate | 1.5 | | | | | |
| 4 | Carnabua Wax | | 1.5 | | | | |
| 5 | HPMC K 100 LVCR | | | 1.5 | | | |
| 6 | Eudragit RLPO | | | | 1.5 | | |
| 7 | Ethyl cellulose N20 | | | | | 1.5 | |
| 8 | Gelucire 50/3 | | | | | | 1.5 |
| 9 | PVP K-90 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 10 | Aerosil P 200 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| 11 | Magnesium Stearate | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 12 | Purified Water | q.s. | q.s. | q.s. | q.s. | q.s. | q.s. |
| | Total | 10 | 10 | 10 | 10 | 10 | 10 |

Initial trials were undertaken to screen polymer not only feasible for extrusion but also provide sustain action from matrix pellets. Hence, *in vitro* dissolution in phosphate buffer pH 6.8 was undertaken to evaluate the same. The different polymers selected are shown in Table 6 and results of *in vitro* dissolution are shown in Fig. 1. Thermal sintering at 80$^{o}$C for 20 minutes was applied wherever wax was used as matrix former for melting and redistribution of wax. From Fig.1 it can be clearly observed that all the polymers as such were inefficient in sustaining the drug release. The initial high release from all the batches is may be due to higher solubility of drug and drug loading which lead to easy diffusion of the drug out of the matrix. Amongst them, the fastest release was shown by batch INH EX 3 containing HPMC K 100 LVCR as release controlling polymer and slowest release was shown by batch INH EX1 containing glyceryl behenate. Hence, batch INH EX 1 was selected from screening studies for further improvement in dissolution in pH 6.8 phosphate buffer. Batch INH EX 6 containing Gelucire 50/3 was dropped as it was not feasible for extrusion.

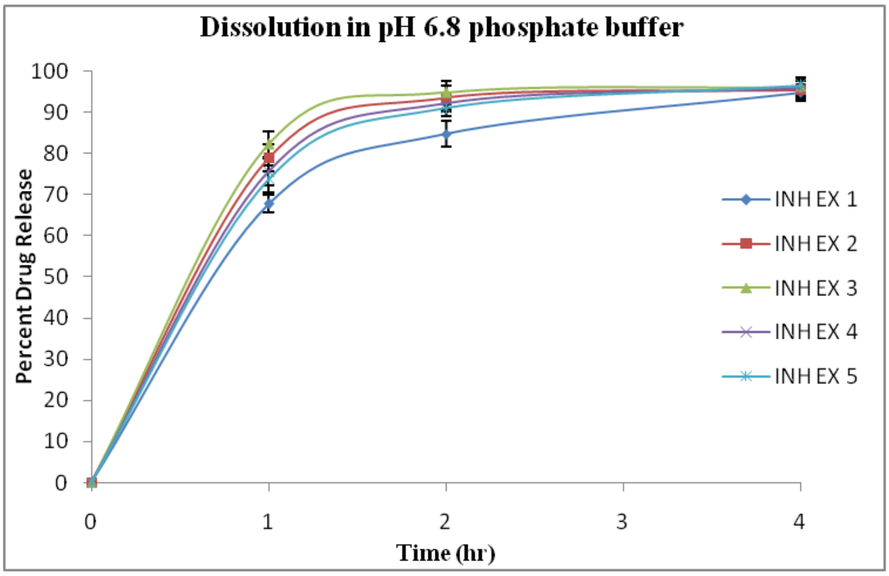


**Fig. 1: Preliminary trials for selection of type of polymer**

Further preliminary trials were undertaken to investigate effect of type of binder on *in vitro* dissolution as depicted in Table 7. The binder concentration depicted in Table 7 was selected based upon preliminary trials conducted to examine extrudability of the damp mass. The results are depicted in Fig. 2.

**Table 7: Preliminary trials for evaluating different types of binders**

| Sr.No. | Ingredients | Batch (%w/w) | | |
|---|---|---|---|---|
| | | INH EX 7 | INH EX 8 | INH EX 9 |
| 1 | Isoniazid | 2 | 2 | 2 |
| 2 | MCC PH 101 | 6 | 6 | 5.9 |
| 3 | Glyceryl behenate | 1.5 | 1.5 | 1.5 |
| 4 | HPC HF | 0.3 | | |
| 5 | Polyox N12K | | 0.3 | |
| 6 | HPMC K4M | | | 0.4 |
| 7 | Talc | 0.1 | 0.1 | 0.1 |
| 8 | Magnesium Stearate | 0.1 | 0.1 | 0.1 |
| | Total | 10 | 10 | 10 |

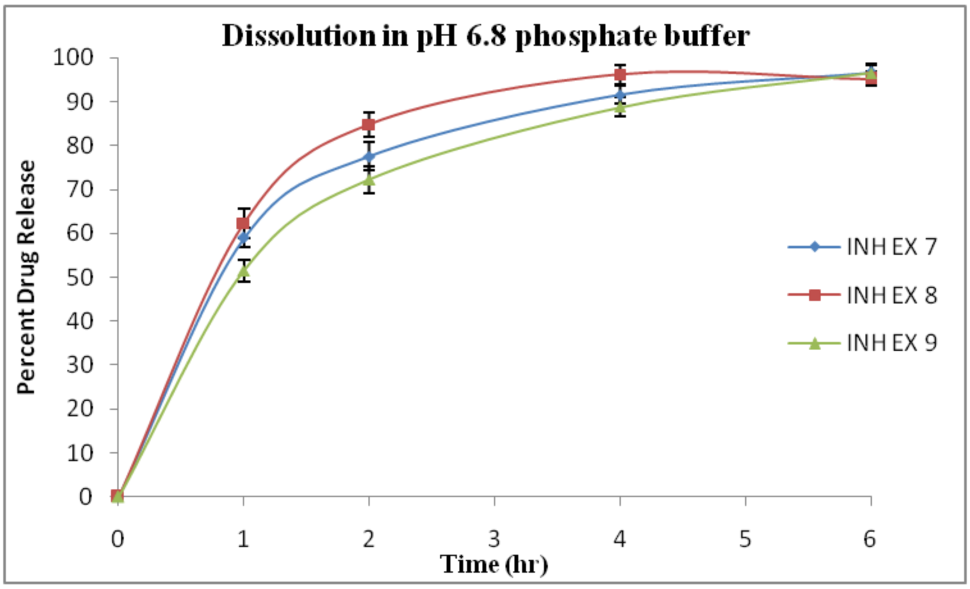


**Fig. 2: Preliminary trials for evaluation of type of binder.**

From the binders screened the maximum slow down in dissolution was observed in batch INH EX 9 containing HPMC K4M as binder. The reason may be stronger binding effect provided by HPMC K4 M as compare to other binders which may be due to its better swelling and redistribution. Hence, HPMC K4M as binder was fixed.

Further trials undertaken involved incorporation of additional polymer in combination with glyceryl behenate to observe its effect on decreasing drug release. Several combinations with ethyl cellulose EC N80 were also investigated. The trials undertaken are depicted in Table 8. The results are displayed in Fig. 3. The results clearly reveal that the batch INH EX 15 containing combination of glyceryl behenate and carbopol 934P was most efficient in retarding drug release giving sustain release for 8 hr. Incorporation of EC into glyceryl behenate was inefficient (INH EX 11 and INH EX 12) in further improvement in retarding the drug release. The reason may be high solubility of the drug. Drug on the surface of the pellets released immediately upon contact with the dissolution medium creating pores and more channels which allow higher ingress of medium and ultimately higher release. On the contrary, carbopol 934 P swell immediately upon contact with dissolution medium pH 6.8 phosphate buffer and forms the comparative thicker gel layer which may be responsible for retarding the drug release.

Combination of carnaubua wax instead of glyceryl behenate with EC N80 and combinations of EC N80 with carbopol 934P were also explored. The results of the same are depicted in Fig. 3 which clearly reveal their inferiority in retarding drug release against combination of glyceryl behenate and carbopol 934P.

**Table 8: Preliminary trials for investigating polymeric combinations.**

| Sr.No. | Ingredients | Batch (%w/w) | | | | |
|---|---|---|---|---|---|---|
| | | INH EX 10 | INH EX 11 | INH EX 12 | INH EX 13 | INH EX 14 |
| 1 | Isoniazid | 2 | 2 | 2 | 2 | 2 |
| 2 | MCC PH 101 | 3.9 | 3.9 | 3.9 | 3.9 | 3.4 |
| 3 | Glyceryl behenate | 1.5 | 1.5 | 1.5 | | |
| 4 | HPMC K4M | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| 5 | Carbopol 934 P | 2 | - | - | - | 2 |
| 6 | Ethyl cellulose N20 | - | 2 | - | - | - |
| 7 | Ethyl cellulose N80 | - | - | 2 | 2 | 2 |
| 8 | Carnabua Wax | - | - | - | 1.5 | - |
| 9 | Talc | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| 10 | Magnesium Stearate | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| | Total | 10 | 10 | 10 | 10 | 10 |

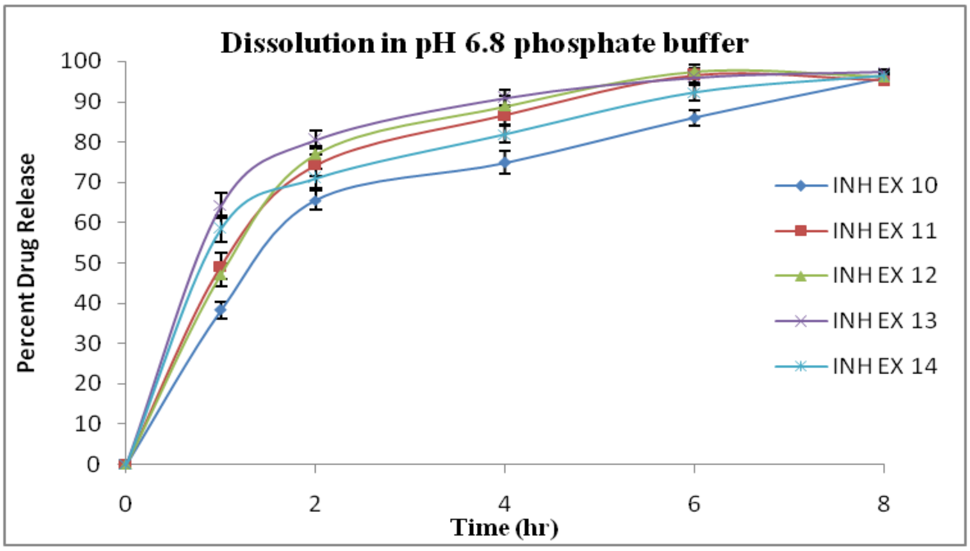


**Fig. 3: Preliminary trials for investigating polymeric combinations.**

In the next trial amount of carbopol 934 P was increased as per maximum level feasible for processing during extrusion and spheronization. The trials are represented in Table 9 and the results are presented in Fig. 4. From Fig. 4, it can be concluded that increase in amount of carbopol 934 P decrease the drug release in the initial hours but was not able to further retard drug release at later hours of dissolution. Overall, no major difference was found on retarding overall drug release on increasing polymer concentration. The reason may be the high solubility of the drug and threshold potential of the polymer as drug release does not result solely from polymer erosion, but also on drug diffusion through the hydrated polymer layers. Probably the high diffusion coefficient of water soluble drug may have quash the effect of higher polymer concentration.

Though no extensive difference in drug release profile was found between INH EX 10 and INH EX 15; batch INH EX 15 was selected as the optimized batch considering its better *in vitro* drug release characteristics in the initial hours. The optimized formulation showed usable yield of 82.30±3.21, pellet size 1065.33±12.33µm, aspect ratio of 0.86±0.015 and abrasion resistance of 0.28%.

**Table 9: Composition batches containing lower and higher amount of polymer Carbopol 934P.**

| Sr.No. | Ingredients | %w/w | mg/capsule | %w/w | mg/capsule |
|---|---|---|---|---|---|
| | | INH EX 10 | INH EX 10 | INH EX 15 | INH EX 15 |
| 1 | Isoniazid | 2 | 75 | 2 | 75 |
| 2 | MCC PH 101 | 3.9 | 146.25 | 3.5 | 131.25 |
| 3 | Glyceryl behenate | 1.5 | 56.25 | 1.5 | 56.25 |
| 4 | HPMC K4M | 0.4 | 15 | 0.4 | 15 |
| 5 | Carbopol 934 P | 2 | 75 | 2.4 | 90 |
| 6 | Talc | 0.1 | 3.75 | 0.1 | 3.75 |
| 7 | Magnesium Stearate | 0.1 | 3.75 | 0.1 | 3.75 |
| 8 | Total | 10 | 375 | 10 | 375 |

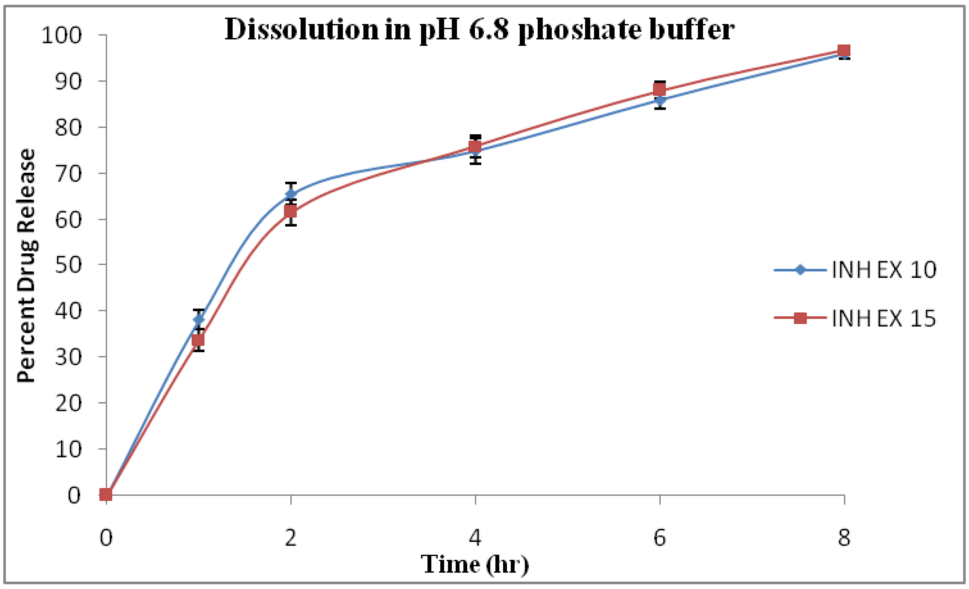


**Fig.4: Effect of Carbopol 934P concentration on *in vitro* dissolution in pH 6.8 phosphate buffer.**

### 5.2.3.5. Curve fitting and release mechanism

Values of adjusted $r^2$, AIC and MSC value are presented in Table 10. The drug release data of the optimized batch INH EX 15 shows a good fit to the first order equation which can be confirmed by comparing the values of adjusted $r^2$ with that of the other models. The lowest AIC value; 22.95, of optimized batch INH EX 15 indicates that first order was the best fit model in describing the dissolution behavior. Similarly, the highest MSC 3.21 of the optimized batch INH EX 15 indicates the same.

The value of constants k1 (44.41) and k2 (-3.47) of Peppas –Sahlin model are displayed in Table 10. k1 denotes relative contribution of drug diffusion to drug release and k2 denotes relative contribution of polymer relaxation to drug release. From Table 10, it is clearly stipulated that diffusion is the predominant mechanism for drug release.

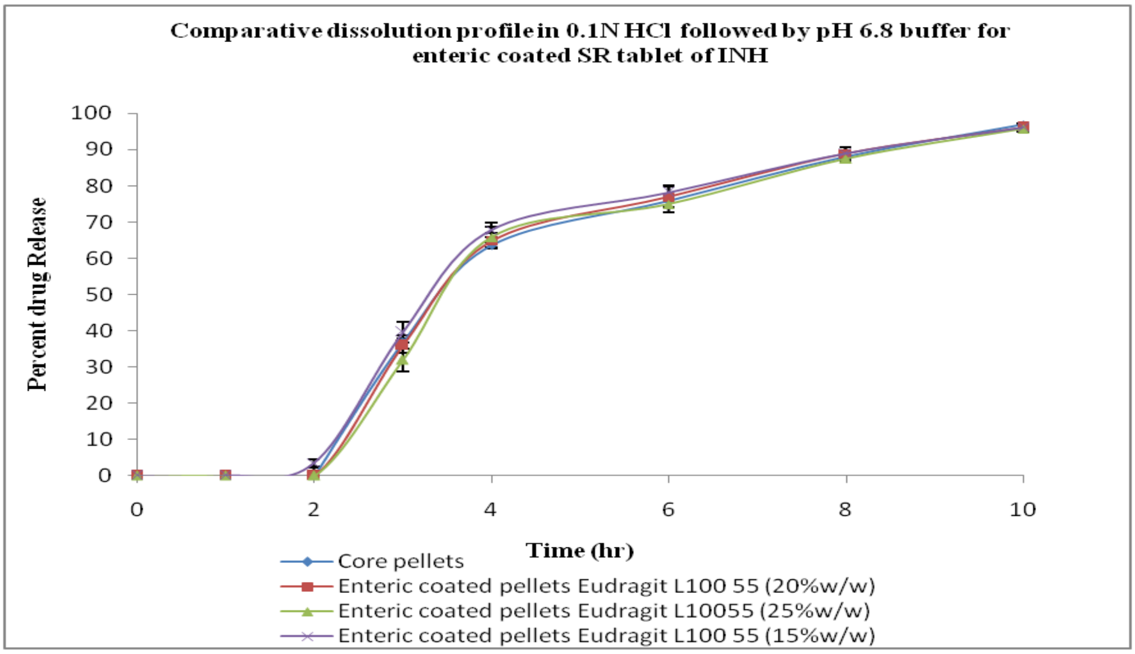
**Table 10: Comparative characteristics of different drug release kinetic models for optimized batch**

| Batch No: | | Zero-order | First order | Higuchi | Hixon-crowell | Hopfenberg | Korsmeyer Peppas | Peppas- Shalin |
|---|---|---|---|---|---|---|---|---|
| INH EX 15 | $r^2$ | 0.1332 | 0.9730 | 0.9361 | 0.9112 | 0.9640 | 0.9437 | 0.9538 |
| | AIC | 40.30 | 22.95 | 27.26 | 28.90 | 24.96 | 27.19 | 26.20 |
| | MSC | -0.26 | 3.21 | 2.35 | 2.02 | 2.81 | 2.36 | 2.56 |
| | | | | | | | 40.97 (k) | 44.41 (k1) |
| | | | | | | | 0.425 (n) | -3.47 (k2) |

### 5.2.3.6. Effect of enteric coating

Here target was set to be zero percentage of drug release in acidic medium as it was anticipated that even small amount of INH release in acidic medium will augment the degradation of RIF.

Enteric coating of 20% w/w was found to be optimum to achieve zero percentage of drug release in 0.1 N hydrochloric acid and similar dissolution profile as of core pellets in pH 6.8 phosphate buffer (Fig. 5). Weight gain of 15% w/w provided similar dissolution profile as that of core pellets in phosphate buffer pH 6.8 but simultaneously drug release of 3.9% (mean value) was obtained in acidic medium. On the contrary, 25% w/w provided zero percentage drug release in acidic medium but slow down dissolution profile of the core pellets during initial hours in pH 6.8 phosphate buffer (Fig. 5).



* Core pellets dissolution shown is only pH 6.8 phosphate buffer.

**Fig. 5: Dissolution of enteric coated SR pellets of INH in 0.1N HCL followed by pH 6.8 phosphate buffer.**

### 5.2.3.7. Packaging and stability studies

The optimized formulation INH EX 15 showed negligible change under the conditions of storage for parameters like appearance, drug content, gastric resistance and *in vitro* drug release. The similarity factor (f2) [9] was employed for comparison of dissolution profiles on each time point. It ranged from 80 to 89. Thus the data suggested that the formulation was stable for under the packaging material selected revealing that it risks it under control and low.

### 5.2.3.8. Risk mitigation and control strategy

Amount of binder as discussed in section 5.3.3.4. was decided to use it at maximum possible concentration based upon feasibility for extrusion and *in vitro* drug release data. Similarly, amount of polymers; glyceryl behenate and Carbopol 934 P were decided to use at optimized concentration based upon feasibility of processing during extrusion and spheronization and *in vitro* drug release data. Risk mitigation strategy is to monitor dissolution in pH 6.8 phosphate buffer. The constraints are as follows; for Q3: $30 < Q3 < 40$, Q6 : $70 < Q6 < 80$ and Q10: $90 < Q10 < 100$.

The enteric coating was also in the high risk category which was optimized as discussed in section 5.3.3.6. Regarding moderate RPN failure modes, packaging was discussed in sections 5.3.3.7. Thermal sintering was kept constant for 20 minutes in all the batches wherever wax was used to exclude its effects on dissolution. Moreover, 20 minutes time was found to be sufficient for melting and redistribution of wax based on preliminary trials. Hence, its risk falls under low category. Fig.6 expresses the FMEA analysis before and after the execution of the control strategy. It was found that RPN of all the possible failure modes were below 20; making them to fall under the low risk category. The final and updated risk based matrix analysis after optimization is depicted in Table 11 and Table 12. It can be clearly observed from the table that risk and impact of formulation variables and unit operations on drug product quality attribute falls under low category. The scalability can be further evaluated from subsequent transfer from lab to pilot and then scale up batch manufacturing. Thus it may be further cultured based on supplementary experience gained during the commercial lifecycle of the production.

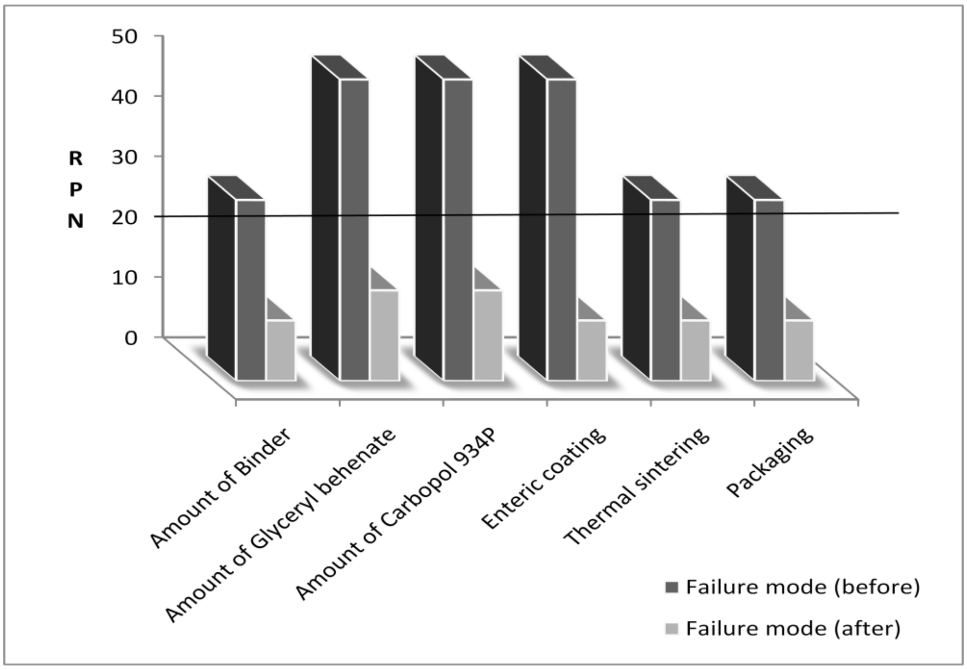


**Fig. 6: FMEA analysis of INH site specific sustain release pellets depicting RPN number of failure mode before and after implementation of control strategy.**

**Table 11: Final and updated risk based matrix analysis for identification of impact of formulation ingredients on drug product attributes.**

| DP CQAs* | Filler | Binder | Polymer | Aerosil P200/ Talc | Magnesium stearate |
|---|---|---|---|---|---|
| Abrasion resistance | Low | Low | Low | Low | Low |
| Shape | Low | Low | Low | | |
| Assay | Low | Low | Low | Low | Low |
| Dissolution in pH 6.8 buffer | Low | Low | Low | Low | Low |
| Gastric Resistance (Drug release in 0.1 N HCL) | Low | Low | Low | Low | Low |

DP CQAs*- Drug product critical quality attributes

**Table 12: Final and updated risk based matrix analysis for identification of impact of unit operations on drug product attributes.**

| DP CQAs* | Sizing | Blending | Granulation | Extrusion | Spheronization | Enteric Coating |
|---|---|---|---|---|---|---|
| Abrasion resistance | Low | Low | Low | Low | Low | Low |
| Shape | Low | Low | Low | Low | Low | Low |
| Assay | Low | Low | Low | Low | Low | Low |
| Dissolution pH 6.8 buffer | Low | Low | Low | Low | Low | Low |
| Gastric Resistance (Drug release in 0.1 N HCL) | Low | Low | Low | Low | Low | Low |

DP CQAs*- Drug product critical quality attributes

## 5.2.4. Conclusion

The research undertaken describes the overall QbD approach along with risk assessment, risk analysis and control strategy to mitigate the risk for development of INH site specific sustained drug delivery. In an endeavor to accomplish the objectives, type of polymer and binder was initially optimized and it was found that glyceryl behenate and HPMC K4M as the most suitable polymer and binder respectively. Finally polymer combination was optimized and it was found that combination of glyceryl behenate with Carbopol 934 P was most efficient in retarding the drug release. The optimized batch exhibited Q3 of 36.84%, Q6 of 75.89%, Q10 of 96.82% and gastric resistance of less than 10%. The RPN of updated risk assessment represents that all the failure modes of FMEA analysis were in low risk category (Fig. 6).

The developed formulation may provide prudently a better substitute for conventional formulations in circumventing its hiccups; improve biopharmaceutical properties, reduce interaction with RIF, providing biphasic release 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 tailored release formulation.

## 5.2.5. References

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