Chapter 5

Segregated drug delivery of two incompatible drugs: Rifampicin and Isoniazid

Chapter 5.1

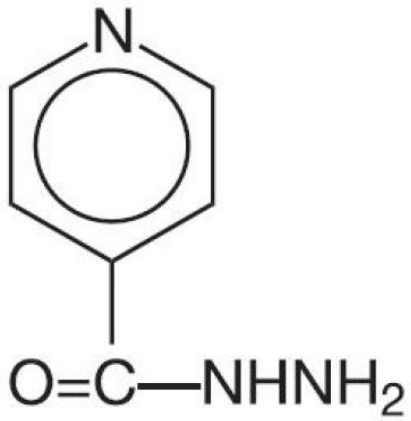
Quality by design enabled formulation development and optimization of enteric coated sustained release Isoniazid tablet

5.1.1. Isoniazid-Drug Profile

Isoniazid (INH) is an organic compound and antibacterial drug use for first line medication for treatment and prevention of tuberculosis [1, 2]. INH shows bactericidal activity against rapidly dividing mycobacteria, and bacteriostatic activity for the mycobacteria which are slow-growing[3].

General Characteristics: [4-8]

- **Molecular Formula:** $C_6H_7N_3O$
- **IUPAC Name:** Isonicotinic acid hydrazide
- **Structure:**



- **Molecular weight:** 137.14 g/mol
- **Appearance and Color:** Colorless or white crystalline powder.
- **Odor:** Odorless.
- **Solubility:** Freely soluble in water, sparingly soluble in alcohol, and slightly soluble in chloroform and ether.
- **Melting point:** 170 ^{o}C to 173^{o}C.
- **Dissociation constants:** Three pKa values: 1.8 (hydrazine nitrogen), 3.5 (pyridine nitrogen) and 10.8 (acidic group).
- **Octanol/Water Partition Coefficient**: log Kow = -0.7.
- **Dose**: 75mg, 100mg, 150 mg and 300 mg.
- **pH of 10% solution** : 6-8.

Mechanism of action

INH is a prodrug that must be activated in *M. tuberculosis* by bacterial catalase-peroxidase enzyme known as KatG. This KatG couples the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex. This complex binds with

InhA; an enoyl-acyl carrier protein reductase and block the action of fatty acid synthase. Thus, INH inhibits the synthesis of mycolic acid which is required for the mycobacterial cell wall [9, 10].

Pharmacokinetics

INH is readily absorbed when administered orally. It reaches peak plasma concentration within 1-2 hr after oral administration. Aluminium containing antacids may interfere with its absorption [5, 11, 12]. It readily diffuses into all body fluids (cerebrospinal, pleural, and ascetic fluids), organs, tissues, and excreta (saliva, sputum, and feces). Moreover, it does not appreciably bound to plasma proteins [5, 12, 13]. INH is primarily metabolized by acetylation and dehydrazination. The rate of acetylation is dependant on genetic factors. The acetylation which is primary route of metabolism converts isoniazid into acetylisoniazid by N-acetyltransferase which is formed in the liver and small intestine. The metabolites do not have anti tubercular activity. [5, 12]. Excretion is primarily renal and over 70% of the dose appears in the urine within 24 hr in patients with normal renal function [12, 14].

Indications and Usage

All forms of tuberculosis in which organisms are susceptible. It is effective for both pulmonary and extrapulmonary tuberculosis [5, 12].

Contraindications

INH is contraindicated in patients who severely develop hypersensitivity reactions. Moreover, it is also contraindicated in alcoholics having impaired liver function or jaundice [5, 15].

Drug Interactions

INH should not be administered with food as it reduces the bioavailability of drug.
It has shown interaction with acetaminophen, carbamezepine, ketoconazole, phenytoin, theophylline, valporate, etc. Additionally, it is reported that it interacts with cytochrome P-450 system where it shows biphasic inhibition induction [5, 9].

Adverse Effects

The most common adverse effects are those which affect central nervous system and liver. Dose dependant peripheral neuropathy is the most common adverse effect related to central nervous system. Other side effects related to central nervous system are convulsions, optic neuritis, memory impairment, etc. Elevated serum transaminase (SGOT; SGPT), bilirubinemia, bilirubinuria, jaundice, etc. are the adverse effects related to the hepatic impairment. Other adverse effects of INH are related to gastrointestinal reactions, hematologic reactions, hypersensitivity reactions, metabolic and endocrine reactions, etc. [5, 9, 15].

5.1.2. Analytical methods

5.1.2.1. Determination of INH in phosphate buffer pH 6.8 by UV spectrophotometry [16]:

Method described by Rastogi *et. al.* was slightly modified and used for estimation of INH for dissolution in initial screening and preliminary trials.

5.1.2.1.1. Preparation of standard stock solutions of INH

INH (100 mg) was dissolved in 30 ml quantity of methanol:phosphate buffer pH 6.8 (50:50%v/v) and volume was made up to 100 ml with methanol:phosphate buffer pH 6.8 (50:50%v/v) to obtain stock solution of 1000µg/ml. An aliquot of 10 ml was accurately taken out with graduated calibrated pipette and further diluted upto 100 ml with phosphate buffer pH 6.8 to obtain working standard solution of 100 µg/ml.

5.1.2.1.2. Preparation of calibration curve of INH

Varying concentrations of INH (5-30 µg/ml) were prepared from 100 µg/ml standard solution using phosphate buffer pH 6.8 as diluent. The absorbances were measured at 263 nm wavelength using UV spectrophotometer (Shimadzu UV1700, Japan) with 1 cm quartz cuvettes and calibration curve was plotted against concentration (µg/ml).
Accuracy and precision were carried out as per ICH guidelines [17]. 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 INH is depicted in Fig. 1. The calibration curve of INH in phosphate buffer pH 6.8, regression analysis equation and correlation coefficient is depicted in Fig. 2.

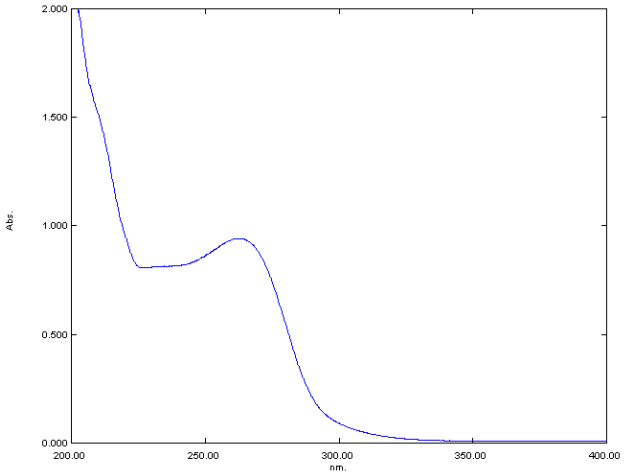


Fig. 1: Reference spectra of INH in phosphate buffer pH 6.8 at 263 nm.

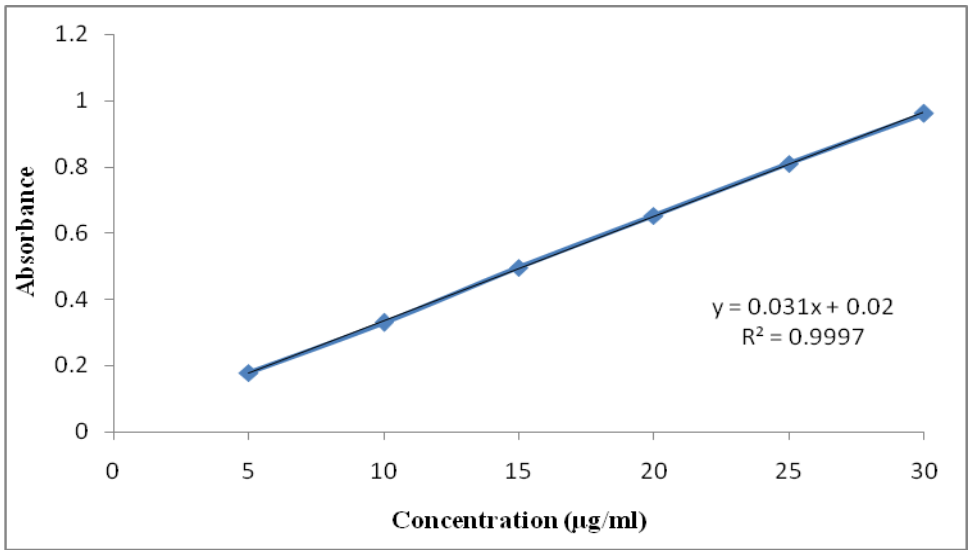


Fig. 2: Calibration curve of INH in phosphate buffer pH 6.8 at 263 nm.

% Recovery:

Table 1: % Recovery for INH

Amount of INH taken Equivalent to (mg)	Amount of Sample Spiked (mg)	Amount of Spiked Sample Recovered	% Recovery
20	12	12.11±0.11	100.92
20	20	19.95±0.15	99.75
20	28	27.92±0.18	99.71

Data are represented as Mean±SD (n=3)

Precision:

Table 2: Precision for INH

	% Relative Standard Deviation
Repeatability	0.91
Intraday	1.33
Interday	1.81

5.1.2.2. Determination of INH in methanol by UV- spectrophotometry:

The method was used for estimation of INH for assay in initial screening and preliminary trials.

5.1.2.2.1. Preparation of standard stock solutions of INH

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

5.1.2.2.2. Preparation of calibration curve of INH

Varying concentrations of INH (2.5-25 µg/ml) were prepared from 100 µg/ml standard solution using methanol as diluent. The absorbances were measured at 263 nm wavelength using UV spectrophotometer (Shimadzu UV 1700, Japan) with 1 cm quartz cuvettes and calibration curve was plotted against concentration (µg/ml).

Accuracy and precision were carried out as per ICH guidelines [17]. For accuracy measurements were taken in triplicate and for precision six determinants were measured. The results of accuracy and precision are depicted in Table 3 and Table 4 respectively No interference of excipients was found at specified detection wavelength.

The reference spectrum of INH in methanol is depicted in Fig. 3. The calibration curve of INH in methanol, regression analysis equation and correlation coefficient is depicted in Fig. 4 respectively.

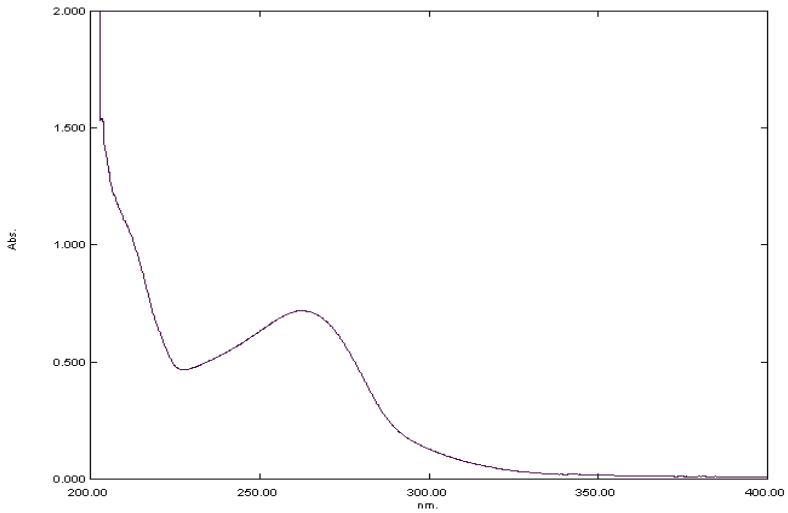


Fig. 3: Reference spectra of INH in methanol at 263 nm.

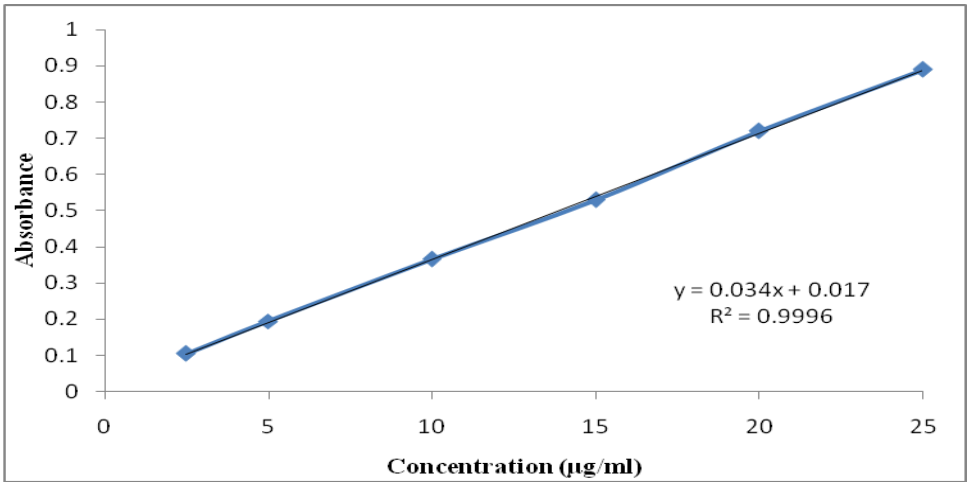


Fig. 4: Calibration curve of INH in methanol at 263 nm.

% Recovery:

Table 3: % Recovery for INH

Amount of INH taken Equivalent to (mg)	Amount of Sample Spiked (mg)	Amount of Spiked Sample Recovered	% Recovery
20	12	11.85±0.05	98.75
20	20	20.21±0.15	101.05
20	28	28.10±0.11	100.36

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

Precision:

Table 4: Precision for INH

	% Relative Standard Deviation
Repeatability	0.85
Intraday	1.21
Interday	1.89

5.1.2.3. Determination of Rifampicin (RIF), INH and 3-Formyl rifamycin (3-FRSV) by reverse phase high performance liquid chromatography method (RP-HPLC) [18, 19]:

The method was used for simultaneous estimation of RIF, INH and 3-FRSV in formulation.

5.1.2.3.1. HPLC instrumentation and conditions

Chromatography was performed on Shimadzu (Shimadzu Corporation, Japan) chromatographic system equipped with Shimadzu LC-20AT pump and Shimadzu SPD-M20A PDA detector. Samples were injected through a Rheodyne 7725 injector valve with fixed loop of 20 µL. The chromatographic separation was achieved using gradient elution as per below mentioned parameters (Table 5 and Table 6). The mobile phase was vacuum filtered through 0.22 µm nylon membrane filter followed by degassing in an ultrasonic bath prior to use. Data acquisition and integration was performed using Spinchrome software (Spincho Biotech, Vadodara). Table 5 represents HPLC parameters for determination of INH, RIF and 3-FRSV and Table 6 represents the gradient programme applied to the method.

Table 5: HPLC parameters for estimation of INH, RIF and 3-FRSV.

Parameter	Condition
Column	Phenomenax C18 (250 mm × 4.6 mm i.d., 5 µm particle size)
Mobile Phase	Buffer solution: pH 6.8 phosphate buffer solution Solution A: Buffer:Acetonitrile (96:4) Solution B: Buffer:Acetonitrile (55:45)
Flow rate	1 ml/min
Detection wavelength	238 nm
Injection volume	20 µl

Table 6: Gradient programme used in the HPLC method

Time (min)	Solution A%	Solution B%	Elution
0	100	0	Equilibration
0-6.5	100	0	Isocratic
6.5-9	100 → 0	0 → 100	Linear gradient
9-21	0	100	Isocratic
21-23	0 → 100	100 → 0	Linear gradient
23-25	100	0	Equilibration

5.1.2.3.2. Preparation of standard stock solutions of INH, RIF and 3-FRSV

INH (100 mg) was dissolved in 50 ml of methanol:phosphate buffer pH 6.8 (4:96%v/v) mixture and volume was made up to 100 ml with methanol:phosphate buffer pH 6.8 (4:96%v/v) mixture to obtain stock solution of 1000µg/ml. An aliquot of 10 ml was accurately taken out with graduated calibrated pipette and further diluted upto 100 ml with phosphate buffer pH 6.8 to obtain working standard solution of 100 µg/ml.

RIF (100 mg) was dissolved in 50 ml of methanol:phosphate buffer pH 6.8 (4:96%v/v) mixture and volume was made up to 100 ml with methanol:phosphate buffer pH 6.8 (4:96%v/v) mixture in 100 ml volumetric flask to obtain stock solution of 1000µg/ml. An aliquot of 10 ml was accurately taken out with graduated calibrated

pipette and further diluted upto 100 ml with phosphate buffer pH 6.8 to obtain working standard solution of 100 µg/ml.

3-FRSV (10 mg) was dissolved in 50 ml of methanol:phosphate buffer pH 6.8 (50:50) and volume was made up to 10 ml with methanol:phosphate buffer pH 6.8 (50:50) to obtain stock solution of 1000µg/ml. An aliquot of 1 ml was accurately taken out with graduated calibrated pipette and further diluted upto 10 ml with phosphate buffer pH 6.8 to obtain working standard solution of 100 µg/ml.

5.1.2.3.3. Preparation of calibration curve of INH

Varying concentrations of INH (2.5-100 µg/ml) were prepared with appropriate dilutions of stock solution with Mobile phase B (Table 5). Calibration graph (Fig. 5) was constructed by plotting area versus concentration of INH and the regression equation was calculated. The calibration curve of INH, regression analysis equation and correlation coefficient is depicted in Fig. 5. System suitability test was performed by injecting six consecutive samples of 30µg/ml during start of method validation. The parameters observed were retention time, tailing factor, theoretical plates and %relative standard deviation (%RSD) of area. The data are represented in Table 7. The accuracy and precision data are represented in Table 8 and Table 9 respectively.

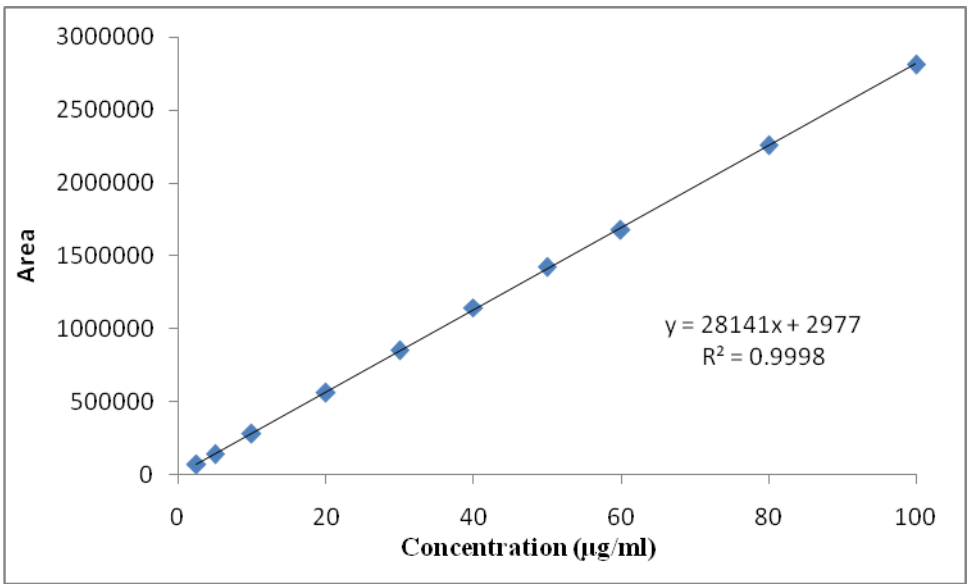


Fig. 5: Calibration curve of INH taken using HPLC method.

System suitability parameters

Table 7: System suitability parameters for estimation of INH

Parameters	Mean±SD
Retention time (min)	6.72±0.02
Asymmetry	1.1±0.06
Theoretical plate	6596.65±28.32
%RSD of area	0.96

% Recovery:

Table 8: % Recovery for INH

Amount of INH Taken Equivalent to (mg)	Amount of Sample Spiked (mg)	Amount of Spiked Sample Recovered	% Recovery
20	12	11.95±0.12	99.58
20	20	20.26±0.10	101.30
20	28	27.90±0.18	99.64

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

Precision:

Table 9: Precision for INH

	% Relative Standard Deviation
Repeatability	0.95
Intraday	1.38
Interday	1.69

The method was also employed for estimation of INH alone in optimization batches. For estimation of INH alone, Mobile phase A was only run in isocratic mode for upto 9 minutes. The validation parameters were found to similar to above parameters and method passed the validation criteria according to ICH Q2 (R1) guideline [17].

5.1.2.3.4. Preparation of calibration curve of RIF

Varying concentrations of RIF (5-200 µg/ml) were prepared with appropriate dilutions of stock solution with Mobile phase B (Table 5). Calibration graph (Fig. 6) was constructed by plotting area versus concentration of RIF and the regression equation was calculated. The calibration curve of RIF, regression analysis equation and correlation coefficient is depicted in Fig. 6. System suitability test was performed by injecting six consecutive samples of 20 µg/ml during start of method validation. The parameters observed were retention time, tailing factor, theoretical plates and %relative standard deviation (%RSD) of area. The data are represented in Table 10. The accuracy and precision data are represented in Table 11 and Table 12 respectively.

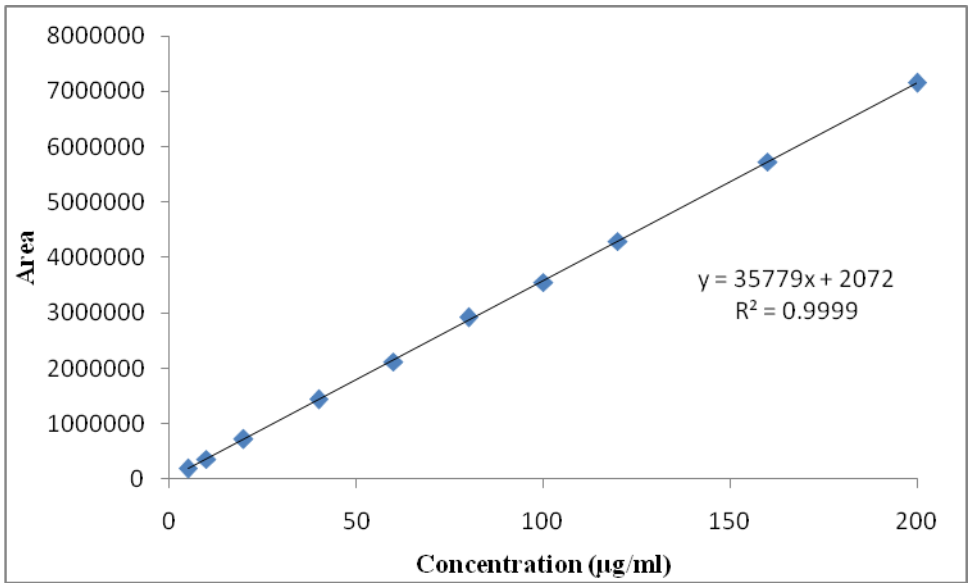


Fig. 6: Calibration curve of RIF taken using HPLC method.

System suitability parameters

Table 10: System suitability parameters for estimation of RIF

Parameters	Mean±SD
Retention time (min)	18.03±0.06
Asymmetry	1.08±0.08
Theoretical plate	73775.62±155.32
%RSD of area	1.12

% Recovery:

Table 11: % Recovery for RIF

Amount of RIF Taken Equivalent to (mg)	Amount of Sample Spiked (mg)	Amount of Spiked Sample Recovered	% Recovery
20	12	12.08±0.12	100.67
20	20	19.90±0.15	99.50
20	28	27.69±0.11	98.89

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

Precision:

Table 12: Precision for RIF

	% Relative Standard Deviation
Repeatability	1.10
Intraday	1.58
Interday	1.90

5.1.2.3.5. Preparation of calibration curve of 3-FRSV

Varying concentrations of 3-FRSV (0.1-10 µg/ml) were prepared with appropriate dilutions of stock solution with Mobile phase B (Table 5). Calibration graph (Fig. 7) was constructed by plotting area versus concentration of 3-FRSV and the regression equation was calculated. The calibration curve of 3-FRSV, regression analysis equation and correlation coefficient is depicted in Fig. 7. System suitability test was performed by injecting six consecutive samples of 2 µg/ml during start of method validation. The parameters observed were retention time, tailing factor, theoretical plates and %relative standard deviation (%RSD) of area. The data are represented in Table 13. The accuracy and precision data are represented in Table 14 and Table 15. The reference chromatogram of INH, RIF and 3-FRSV carried out by gradient HPLC method is depicted in Fig. 8. The limit of detection and limit of quantification were found out to be 0.0095 µg/ml and 0.032 µg/ml respectively The peak purity for 3-FRSV was found out to be 99.69%.

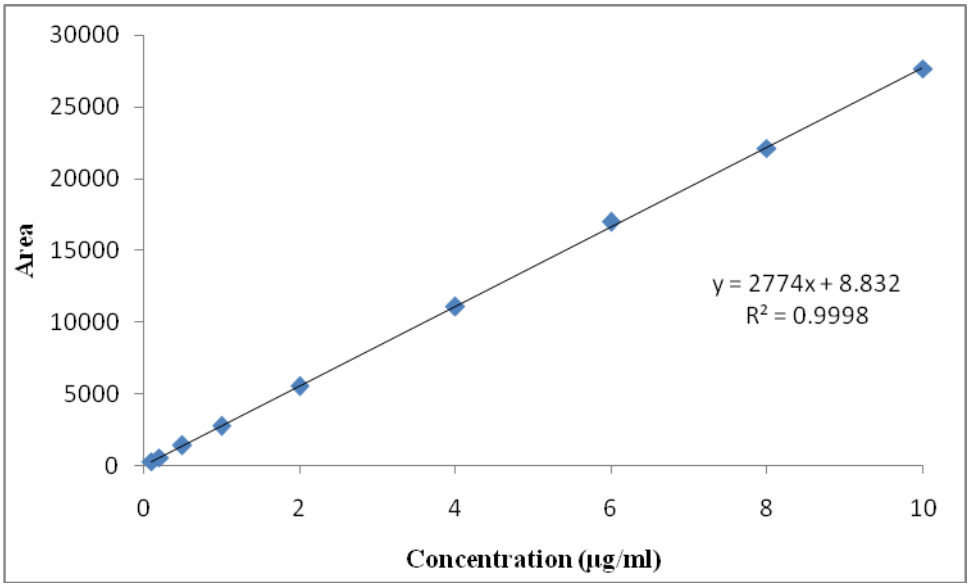


Fig. 7: Calibration curve of 3-FRSV taken using HPLC method.

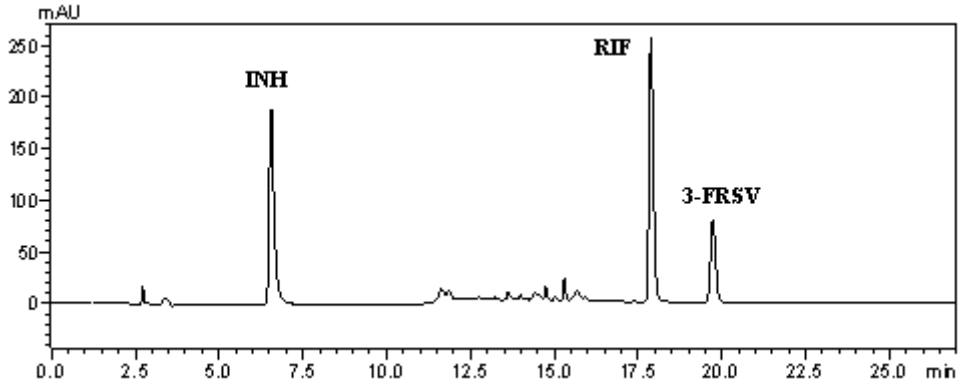


Fig. 8: Reference chromatogram of INH, RIF and 3-FRSV obtained by performed HPLC method.

System suitability parameters

Table 13: System suitability parameters for estimation of 3-FRSV

Parameters	Mean±SD
Retention time (min)	20.69±0.08
Asymmetry	1.11±0.05
Theoretical plate	55982.21±120.55
%RSD of area	1.23

% Recovery:

Table 14: % Recovery for 3-FRSV

Amount of 3-FRSV Taken Equivalent to (mg)	Amount of Sample Spiked (mg)	Amount of Spiked Sample Recovered	% Recovery
2	1.2	1.216±0.006	101.33
2	2	1.978±0.012	98.90
2	2.8	2.819±0.11	100.68

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

Precision:

Table 15: Precision for 3-FRSV

	% Relative Standard Deviation
Repeatability	1.28
Intraday	1.63
Interday	1.85

5.1.3. Methods

5.1.3.1. Quality Target Product Profile (QTPP) of INH site specific sustained release tablet

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 [20]. 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 [21]. The International conference of harmonization (ICH) Q8 (R2) [22] recapitulates them as quality 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, purpose of product, its key desired quality attributes, manufacturing methodology, etc. The anticipated QTPP depends upon scientific and nonscientific considerations [22, 23].

5.1.3.2. 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 [24]. ICH Q9 [25] 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" [25]. 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.

5.1.3.3. 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 [23]. 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.

5.1.3.4. Drug excipient compatibility

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

5.1.3.4.1. 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.

5.1.3.4.2. 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 were investigated using KBr disk method. In brief procedure involved mixing of total of 2% (w/w) of the sample with respect to the potassium bromide (KBr; S. D. Fine Chem Ltd., Mumbai, India) disk. 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.

5.1.3.5. Preparation of INH sustained release core tablets

Initial screening and preliminary trials were carried out using by direct compression method to investigate its feasibility in achieving desired sustained release profile. Subsequent trials were undertaken employing wet granulation technique as granulation method followed by compression to obtain tablet.

5.1.3.5.1. Procedure for preparation of INH tablet by direct compression method.

Briefly, accurately weighed quantity of INH (75 mg/tab), microcrystalline cellulose (MCC PH 102), Polyox WSR 303/Polyox N12K, ethyl cellulose (EC N10)/ hydroxyl propyl cellulose (HPC HF) were sifted through ASTM # 30 (Jayant Scientific Industries, Mumbai, India) and talc sifted through ASTM 60# were physically mixed for about 10 min. Then colloidal silicon dioxide and Butylated hydroxyl toluene (BHT) were sifted through ASTM sieve # 60 and added as a glidant and anti-oxidant respectively 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 shallow concave punches of 7.0 mm diameter to a target weight of 150 mg / tab.

5.1.3.5.2. Procedure for preparation of INH tablet employing wet granulation technique as granulation method

Briefly, accurately weighed quantity of INH (75 mg/tab), MCC PH 101, talc (as an anti-static agent), colloidal silicon dioxide and binder, EC/ HPC/Povidone K-30 (PVP K-30) were sifted through sieve ASTM # 30 (Wire metal GMP products, Mumbai, India) and physically mixed for 10 min. The premix was wet granulated using isopropyl alcohol (IPA) for EC and using purified water was HPC/PVP K30 and dried in hot air oven at 55^0C (Shree Kailash Industries, India). To the dried granules; MCC PH 102, Polyox WSR 303 (previously sifted through sieve ASTM # 30) and BHT (previously sifted through ASTM sieve 60 #) were added and blended for 10 min. Then colloidal silicon dioxide (as glidant, previously sifted through ASTM sieve 60 #) was added and blended for 10 min. Finally, magnesium stearate (as lubricant, previously sifted through ASTM sieve 60 #) was added and blended for 3 min. The homogeneous blend was compressed on an eight station automatic rotary tablet machine (JM-8, General Machinery Co., Mumbai, India) equipped with shallow concave punches of 7.0 mm diameter to a target weight of 150 mg/tab.

5.1.3.6. Seal coating on optimized core tablet

Seal coat on core tablets 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 perforated pan coating apparatus (Solace Engineers (Mktg) Pvt. Ltd., Vadodara, Gujarat) to achieve 3% weight gain. The process conditions were pre-warming of the tablets at 40 ^{o}C for 10 min; spray nozzle diameter, 1mm; atomizing air pressure, 1-1.2 bar; inlet air temperature, 50-55^{o}C; product temperature 38-42^{o}C; spray rate, 5-8 gm/min; pan rpm 6-8, post-drying at 40 ^{o}C for 30 min. The coating was performed to achieve weight gain of 3% w/w.

5.1.3.7. Enteric coating on seal coated tablet

Enteric coating of tablets was done using perforated pan coating apparatus (Solace Engineers (Mktg) Pvt. Ltd., Vadodara, Gujarat) 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 tablets at 40° C temperature for 10 minutes, inlet air temperature (40-50° C), bed temperature (29 - 31° C) atomizing air pressure (1-1.2 bar), rotating speed of pan (7-10 rpm) and spray rate (4-6 gm/min) were adjusted. After finishing of the coating, tablets were kept in the pan at 40° C for curing upto 30 minutes. The coating was performed to achieve three different weight gains viz. 8%w/w, 10%w/w and 12%w/w of average tablet weight.

5.1.3.8. Physical characterization of the tablets

The compressed core tablets were subjected to various physical investigations like appearance, weight variation, hardness, friability and drug content. The weight variation was carried out on 20 tablets using electronic balance (Shimadzu AX 120, Japan). Tablet hardness was determined using minimum 6 tablets for each batch with dial type tablet hardness tester (Scientific Engineering Corporation, Delhi, India). Friability was determined by Friabilator (VFT-2D, Veego Instruments Co., Mumbai, India) for 4 min at 25 rpm. Coated tablet was subjected to investigations like appearance, percentage weight gain and drug content. Drug content measurement was done in triplicate using developed UV-spectrophotometry and RP-HPLC method as discussed in section 5.1.2. Percentage weight gain was computed using following Eq.2 [26].

$$\%\text{Weight gain} = (Wt_a - Wt_b)/ Wt_b * 100 \quad (2)$$

where Wt_a – Weight of tablet after coating.

Wt_b –Weight of tablet before coating

5.1.3.9. *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 tablets [27]. Dissolution profiling and data were recorded at 1, 2, 3, 4, 6, 8, and 10 h. For optimization of core tablets 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 was measured by UV-spectrophotometry and RP-HPLC as described in section 5.1.2.

5.1.3.10. 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 [28], first order release kinetics [29], Higuchi's square root of time equation [30], Baker-Lonsdale equation [31], Hopfenberg equation [28, 32] Hixson - Crowell's cube root of time equation [33], Weibull [34] and Korsmeyer-Peppas power law equation [35,36]. 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 [37].

Additionally, the data were also fitted into Peppas- Sahlin model to understand drug release mechanism. [38, 39].

5.1.3.10.1. Akaike information criterion (AIC)

The sum of the squared residuals (SSR) can be found out to distinguish the models that described the best fit. But greater number of model parameters 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. [37, 40]. The AIC is dependent on the magnitude of the data and on the number of data points. The AIC is defined as

$$AIC = n.\ln(WSS) + 2.p \qquad (3)$$

where n is the number of data points, WSS is the weighted sum of squares and p is the number of parameters in the model. To distinguish amongst various models for better fit, the model with a relative lower AIC value indicates better fit. It should be noted that the weighting scheme used in each model must be the same when a comparison is made [37].

5.1.3.10.2. Model selection criteria (MSC)

The MSC is an another fascinating statistical criterion for model selection and evaluating goodness of fit [37]; it is defined as:

$$MSC = \ln\left[\frac{\sum_{i=1}^{n} wi \ . (yi\_obs - \overline{y}\_obs)^2}{\sum_{i=1}^{n} wi \ . (yi\_obs - yi\_pre)^2} - \frac{2p}{n}\right] \quad (4)$$

where *wi* is the weighting factor, which is generally equal to 1 for fitting dissolution data, *yi_obs* is the i^{th} observed *y* value, *yi_pre* is the i^{th} predicted *y* value, *y_obs* is the mean of all observed *y* data points, *n* is the number of data points and *p* is number of parameters. The MSC is a modified reciprocal form of the AIC and has been normalized so that it is independent of the scaling of the data points. To distinguish amongst various models for better fit, the model with the largest MSC value will be the most suitable. Usually, a MSC value of more than two to three signifies a good fit [37].

5.1.3.11. 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 [41]. 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).

5.1.3.11.1. Capability indices

Capability indices Cp, CPU, CPL and Cpk were computed for potential within capability and Pp, PPU, PPL and Ppk for overall capability respectively [42, 43]. The 3-σ standard deviation variation was everywhere considered for relating process spread to specification spread. Capability index Cp is the ratio of the specification spread (Upper Specification Limit (USL) – Lower Specification Limit (LSL)) to the process spread. It is sometimes considered as simply process precision. It does not consider centering of the process but rather focuses on dispersion of the process [44-46]. To overcome the pitfall of Cp, Cpk was introduced which takes into consideration both process departure from the centre and extent of the process variance. Thus it is a useful marker of how actually your process is performing. [45,

46]. One major limitation of Cpk is that it does not account characteristics whenever one specifies target value. Cpm overcomes this drawback and it is the ratio of the specification spread (USL - LSL) to the square root of the mean squared deviation from the target. It inspects the process spread and the change of the mean from the target and compares them with the specification spread. Any observation that is away from the target will increase the standard deviation [47]. CPU/CPL is a capability index related to one sided specification only. It compares distance between upper specification limit and process mean to the upper half width of distribution. It considers both process spread and process center [45, 48]. Ideally, the process to be centered within specification limits, Cp and Cpk should be approximately equal [45, 49]. Similarly Pp, PPU, PPL and Ppk are indicators for overall capability indices [42, 43].

5.1.3.12. Packaging and stability study

The optimized batch was subjected to short term stability testing according to the ICH guidelines [50]. 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. 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 and *in vitro* drug release.

5.1.4. Results and Discussion

5.1.4.1. QTPP of INH site specific sustained release tablet

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 [21, 51]. The parameters that will be focused in our study were chosen and enlisted as QTPP for INH enteric coated sustain release 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 INH site specific sustained release tablet is highlighted in Table 16. The depicted QTPP will lay down the basis for determining CQA.

Table 16: QTPP of INH site specific sustained release tablet

QTPP element	Target	Justification
Dosage form	Enteric coated sustain release tablet which gives no release in 0.1N HCl but gives sustain action in pH 6.8 buffer	Tablet because commonly accepted unit solid oral dosage form. Enteric coated because to prevent INH dependant RIF degradation in acidic media by segregating its zone of delivery. 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 RIF (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	Physical Attributes	No physical defects like chipping, lamination, capping, etc. in core tablet and no coating defects in coated tablet
	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).
	Gastric resistance/Drug release in 0.1NHCl	Less than 10%
	Dissolution in pH 6.8 buffer	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.

5.1.4.2. 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 [21, 25]. Table 17 describes risk assessment of INH site specific sustained release tablet with their respective justifications. From the Table 17 it can be revealed that hardness, 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 18 and 19 respectively. Further quantitative risk analysis was carried out using FMEA method to select the most critical factors which needed further investigation.

Table 17: Risk assessment of INH enteric coated sustained release 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 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 of core tablets	Not more than 0.5%w/w	No	A target is set according to the compendial requirement (NMT1%) Narrower target is set (NMT 0.5%) for tablet coating purpose. As friability will not impact patient safety or efficacy, this not critical.
	Hardness	Depending upon drug release profile and polymer concentration	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 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 and will have major percentage of tablet weight (approximately 50%), there are less likely chances of variation in content uniformity. Therefore it is not critical.
Dissolution in pH 6.8		Biphasic release: Initial burst release	Yes	Both formulation and process variables can greatly impact the dissolution profile. Thus it will be observed throughout

buffer		within first hour and thereafter sustained release for 8 hours.		the formulation development and optimization. Moreover failure to meet this specification will have direct impact on bioavailability and bioequivalence. Hence, it is critical.
Gastric Resistance		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 18: Initial risk based matrix analysis for identification of impact of formulation ingredients on drug product attributes.

DP CQAs*	Polymer	Binder	Filler	Aerosil 200	Magnesium stearate
Hardness	Low	Low	Medium	Low	Low
Assay	Low	Low	Low	Low	Low
Dissolution in pH 6.8 buffer	High	High	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 19: Initial risk based matrix analysis for identification of impact of unit operations on drug product attributes.

DP CQAs*	Sizing	Blending	Granulation	Compression	Granule sizing	Enteric Coating
Hardness	Low	Low	Low	High	Medium	Low
Assay	Low	Low	Low	Low	Low	Low
Dissolution pH 6.8 buffer	Low	Low	Low	High	Medium	Low
Gastric Resistance (Drug release in 0.1 N HCL)	Low	Low	Low	Low	Low	High

DP CQAs*- Drug product critical quality attributes

5.1.4.3. FMEA approach for risk analysis

The FMEA method was used to perform the quantitative risk assessment, which could identify the modes that have 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 [52]. Table 20 enlists the factors that were considered in development of INH site specific extended release tablet while performing FMEA. From the Table 20, it is clearly stipulated that amount of polymer, hardness, amount of binder and enteric coating have RPN ≥ 40; thus requiring through investigation and optimization. Thus, the optimization of three main factors that affect the core tablet formulation i.e. amount of polymer, hardness and amount of binder was be done using design of experiment for establishing design space. The enteric coating RPN also falls under high risk category and its optimization is discussed in its respective section 5.1.4.11. Granule sizes and packaging RPN fall under moderate risk category and are also discussed in their respective sections 5.1.4.10. and 5.1.4.13.

Table 20: 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
Hardness	Inadequate hardness and its range	drug release and friability	5	Machine failure, operator's error, excipient selection	4	Hardness tester, friability testing, dissolution	2	40
Amount of Polymer	Improper concentration	Drug release	5	Improper concentration	5	Dissolution	2	50
Amount of binder (ethyl cellulose)	Improper concentration	Drug release	5	Improper concentration	5	Dissolution	2	50
Enteric coating	Improper coating	Gastric resistance	5	Improper weight gain, coating uniformity	5	Gastric resistance, dissolution	2	50
Granule sizes	Improper size	Drug release	5	Improper size	2	Dissolution	3	30
Packaging	Insufficient to protect drug from temperature, humidity and shipping.	Stability	5	Packaging material	3	Assay, dissolution, hardness	2	30

5.1.4.4. Preliminary trials for evaluating feasibility of direct compression method

Before starting preliminary trials, all the excipients were checked for compatibility with drug by DSC and FT-IR studies. The results revealed all the excipients used in this study were compatible with INH.

Polyox was selected as sustained release polymer based on literature as it swells very fast and is effective in prevention of high burst release of highly water soluble drugs [53, 54]. Initially a high and medium viscosity grade was selected for preliminary trials considering the high proportion of INH in formulation and also its high solubility. Three different concentrations of both Polyox types were evaluated as shown in Table 21 (Batch IDC 1 to IDC 6). In another next two trials, dry binders were incorporated; one of which is hydrophobic; EC N10 (IDC 7) and other one hydrophilic; HPC HF (IDC8) to investigate its effect on retarding dissolution in pH

6.8 phosphate buffer. The composition of the preliminary batches and results of the same is depicted in Table 21.

Table 21: Preliminary trials for evaluating feasibility of direct compression method

		IDC1	IDC 2	IDC 3	IDC 4	IDC 5	IDC 6	IDC 7	IDC 8
Sr. No.	**Ingredients**	**mg/tablet**							
1	Isoniazid	75	75	75	75	75	75	75	75
	Polymer								
2	Polyox WSR 303	30	45	60				30	30
3	Polyox WSR N12 K				30	45	60		
	Filler								
4	MCC PH 102	42.65	27.65	12.65	42.65	27.65	12.65	26.9	26.9
	Dry binder								
5	Ethyl Cellulose N 10							15	
6	HPC HF								15
	Antioxidants								
7	Butylated hydroxy toluene (BHT)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Glidant								
8	Talc							0.75	0.75
9	Colloidal silicon dioxide (Aerosil P 200)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	Lubricant								
10	Magnesium stearate	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
	Total	150	150	150	150	150	150	150	150
	Physical Evaluation								
	Hardness (Kp) (n=6)	6.2-7.8	5.5-6.8	5.1-6	6.0-7.6	6-6.8	5-5.8	5.3-6.3	5.5-6.9
	Friability	Nil	0.11	0.23	0.12	0.15	0.28	0.15	0.18
	Assay (n=3)	101.6± 0.9	102.5±1.1	98.6±1.2	100.9± 1.2	98.5±0.8	99.2±1.2	101.8±0.8	98.2±0.9
	Weight Variation %SD (n=20)	±2.6	±1.2	±1.1	±1.6	±1.8	±1.2	±1.9	±1.8
	Dissolution in 900 ml, phosphate buffer pH 6.8,paddle, 50 rpm								
	Time in hr	**Percent drug released**							
	1	38.8±1.6	36.1±2.1	35.5±2.6	51.5±2.3	53.2±2.6	50.8±1.5	37.9±1.2	35.7±1.8
	2	48.8±2.1	46.4±2.8	45.7±2.9	70.3±1.6	72.1±1.8	72.3±2.1	47.4±2.9	44.9±2.8
	4	76.1±2.3	78.3±1.9	74.3±1.5	91.2±2.1	88.2±1.9	90.8±1.8	78.8±2.3	75.1±3.5
	6	95.2±1.9	92.6±2.5	94.2±3.2	97.6±1.2	93.2±1.1	95.5±1.5	92.5±3.3	96.1±1.8

Batch IDC 1 to IDC 3 contains increasing amount of Polyox WSR 303 as sustained release polymer. Results of the same are displayed in Table 21. The physical parameters like friability, assay and weight variation were found to be satisfactory. The *in vitro* drug release reveal no significant difference at all the time points with more than 90% drug release was observed at 6 hr time point suggesting incapable of release upto 8 hr in pH 6.8 phosphate buffer. The reason may be the threshold level of retardation of drug release rate by polymer as drug release does not result exclusively from polymer erosion, but also on drug diffusion through the hydrated polymer layers. Another major reason may be the high solubility of drug and drug loading. Probably, the high diffusion co-efficient of water soluble drug may be also one of the factors responsible for higher release. Additionally, for batch containing 60 mg/tab (40%w/w of tablet), sticking was observed during compression which may be due to high concentration of Polyox WSR 303. Batches IDC 4 to IDC 6 contains increasing amount of Polyox WSR N12-K. They showed faster release than batches IDC1 to IDC3 due to lower molecular weight than Polyox WSR 303 with more than 85% drug release in 4 hr suggesting incapable of sustaining drug release. Effect of increasing amount of polymer had no significant effect on *in vitro* drug release and results were similar to batches IDC 1 to IDC3. Hence, from these preliminary trials, batch IDC 1 was selected for further processing and improvisation.

In the subsequent trials, batch IDC 7 and IDC 8 dry binder of diverse type were included in formula of batch IDC 1 for investigating its effect on improvement of drug release. The results are displayed in Table 21. Results reveal that incorporation of these binders were inefficient in slowing down the drug release as compare to previous batches containing no binder.

Hence, future trials were planned to incorporate binder into granulation step and investigate its effect on improvement of drug release profiles. For these; binders with different type of nature; hydrophilic and hydrophobic were evaluated. PVP K-30 and HPC HF were selected from class of hydrophilic binder and EC N10 was selected from class of hydrophobic binder. In case of hydrophilic binder, purified water was used as granulating solvent and for hydrophobic binder isopropyl alcohol (IPA) was used as granulating solvent. As discussed earlier, amount of Polyox WSR 303 was keep at 30 mg/tab (20%w/w of tablet) for further trials as further increase did not

resulted in significant improvement in dissolution and higher concentration of 60 mg/tab created some processing issues. The preliminary trials undertaken for selection of type of binder employing wet granulation technique is depicted in Table 22. The percent drug release profile in pH 6.8 phosphate buffer in depicted in Fig. 9.

Moreover, on basis of its minimum inhibitory concentration, volume of distribution and fraction bioavailable, a minimum of approximately 16 % should be released as initial loading dose theoretically. It was not an issue here as all the batches showed higher drug release due to higher drug dose and solubility.

Table 22: Preliminary trials for selection of type of binder in wet granulation method.

Sr. No.	Ingredients	IWG 1	IWG 2	IWG 3	IWG 4
1	Isoniazid	75	75	75	75
	Intragranular (Premix)				
2	MCC PH 101	24.9	24.9	24.9	24.9
3	Talc	0.75	0.75	0.75	0.75
4	Colloidal silicon dioxide (Aerosil P 200)	0.75	0.75	0.75	0.75
5	Ethyl Cellulose N 10		7.5		
6	HPC HF			7.5	
7	PVP K-30				7.5
	Binder solution				
8	Ethyl Cellulose (N10)	7.5			
9	IPA (q.s.)	q.s.	q.s.		
10	Purified Water (q.s.)			q.s.	q.s.
	Extra granular				
11	MCC PH 102	9.5	9.5	9.5	9.5
12	Polyox WSR 303	30	30	30	30
13	Butylated hydroxy toluene (BHT)	0.1	0.1	0.1	0.1
	Glidant				
14	Colloidal silicon dioxide (Aerosil P 200)	0.75	0.75	0.75	0.75
	Lubricant				
15	Magnesium stearate	0.75	0.75	0.75	0.75
	Total	150	150	150	150
	Physical Evaluation				
	Hardness (Kp)- (n=6)	-	6.2-8.9	6.5-8.8	5.8 -8.5
	Friability	-	0.18	0.1	0.19
	Assay (n=3)	-	100.6± 1.1	101.5±1.5	99.6±1.2
	Weight Variation %SD (n=20)	-	± 1.8	± 1.5	± 2.1

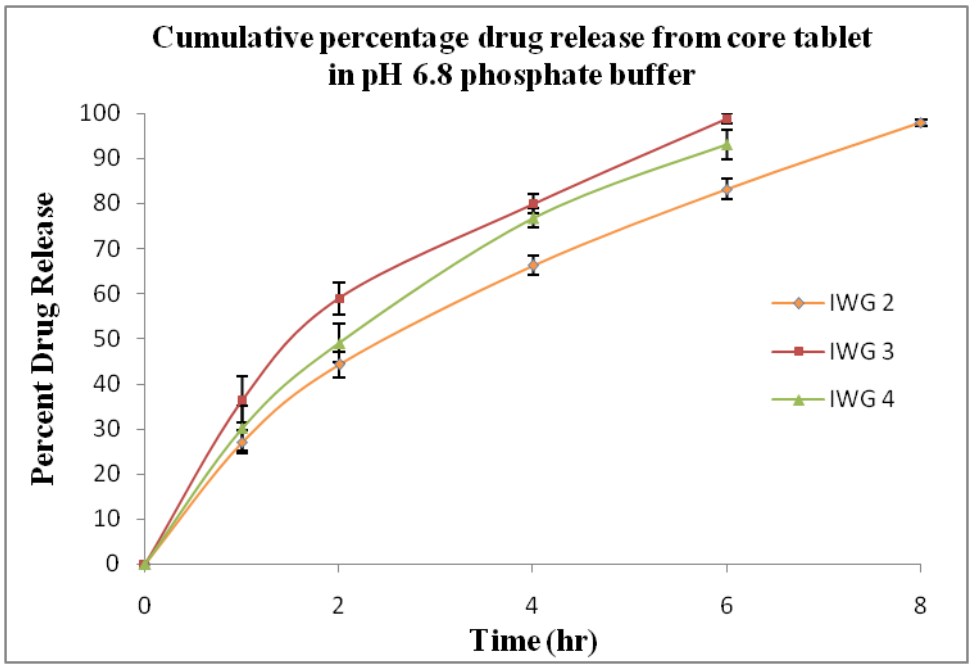


Fig. 9: Cumulative percent drug release of preliminary trials in pH 6.8 phosphate buffer

For batch IWG 1, binder solution was prepared by dissolving EC in IPA. This binder solution was used to granulate the premix blend. It was anticipated that using binder solution effect of binder would be stronger than adding it into premix. But the granulation end point was achieved early consuming only part of binder solution and part of binder solution remained unused. Hence, the trial was dropped and in subsequent trials binder was added in the premix for all type of binders.

The physical characteristics of the tablets prepared for batches IWG 2 to IWG 4 were found to be satisfactory as shown in Table 22. The percent drug release in depicted in Fig. 9. From Fig. 9, it can be revealed that batch IWG 3 and IWG 4 containing HPC and PVP K 30respectively showed higher release with more than 90% drug releasing at the end of 6 hr in pH 6.8 phosphate buffer. On the contrary, EC N10 slowed down the overall release profile showing initial higher release and thereafter sustained release upto 8 hr which was desirable.

The reason may be their respective binding mechanisms together with its interaction with water and IPA (PVP K 30, HPC and EC respectively) i.e. solution properties which ultimately affected granulation rate and granule properties significantly. PVP K 30 absorbed water and may underwent a phase transition to the rubbery/solution state

from the glassy state [55, 56].Thus based on entanglement theory, PVP K30 chains can be entangled to form a physical network which would have resulted in adhesive strength for binding. Similarly, HPC may underwent phase transition from glassy to rubbery/solution state during granulation to exhibit adhesive strength for binding [56, 57]. While in case of EC it is hypothesized that it dissolved on addition of IPA and it exhibits membrane coating like effect. Now as EC is hydrophobic in nature and INH is hydrophilic, the hydrophobic-hydrophilic interaction is more efficient in preventing drug release as compared to PVP K-30/HPC. Another mechanism might be as EC is hydrophobic it has low affinity for water. Thus it retards incursion of dissolution medium and ultimately cause delay in drug release. Hence, the dynamics of phase transition of binders in the wet granulation process together with solution properties of binding solvent may be involved in retarding the drug release. The overall high initial drug release in both the batches might be due to high solubility of the drug in the medium and secondary due to high drug loading.

Thus batch IWG 2 was selected as prototype formula and optimization was carried out on this formula. Moreover, hardness, amount of Polyox WSR 303 (Polymer) and amount of binder were under high risk category (RPN ≥40) which warranted their detail investigation. Thus their optimization was done using 2^3 full factorial design as optimization technique. For binder, current level (5%w/w of tablet) and higher level was selected (7.5% w/w of tablet) to investigate effect of increasing binder concentration on further retardation of drug release. Similarly amount of Polyox WSR 303 was also increased to investigate the same. For hardness, two levels were selected; higher hardness level range and lower hardness level to investigate its effect on percent drug release and for establishing design space.

5.1.4.5. 2^3 full factorial design for optimization of core tablet

A 2^3 factorial design with 3 factors, 2 levels, and 8 runs was selected for the optimization study independent and dependent variables with their constraints are listed in Table 23. Perceent drug release in pH 6.8 phosphate buffer at 1st hour (Q3) , 2nd hour (Q4), 4th hour (Q6) and 8th hour (Q10) were selected as dependant variables. For predicting the optimal region, the linear polynomial equation generated for the variables was explained as follows (Eq. 5):

$$Y = \beta_0 + \sum \beta ixi + \sum \beta ijxixj \qquad (5)$$

Where *Y* is the predicted response, β_0 is model constant/ coefficient, βi is the linear 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 design of experiment 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 runs 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 [58] and Q^2 [59].

Table 23: Formulation variables and their levels for 2^3 full factorial design.

Factors	Coded levels	Actual Levels
A: Amount of Polyox WSR 303 (mg/tab)	-1	30
	1	45
B: Hardness (Kp)	-1	3-5
	1	6-9
C:Amount of ethyl cellulose (mg/tab)	-1	7.5
	1	11.25
Responses (Percent drug release in pH 6.8 buffer)	Constraints	
Q3:Percent drug released in 1 hr	20%≤Q3≤30%	
Q4:Percent drug released in 2 hr	40%≤Q4≤50%	
Q6:Percent drug released in 4 hr	60%≤Q6≤75%	
Q10:Percent drug released in 8 hr	90%≤Q10≤100%	

5.1.4.6. Physical characterization of tablets

Physical appearance, weight variation and assay of all the formulations of core tablets were found to be satisfactory. Hardness was studied in two ranges 3-5 Kp for lower level and 6–9 Kp for higher level. Friability of the core tablets for both the level range was found to be less than 0.5% (w/w).

5.1.4.7. Effect of factors on the responses

5.1.4.7.1. Q 3: Percent drug released in 1 hr in pH 6.8 phosphate buffer

Results of the measured response for 2^3 full factorial design are displayed in Table 24. Regression coefficients and ANOVA results of response variables are shown in Table 25. From the results, it can be concluded that hardness was the most influencing factor affecting negatively (Table 25) in initial burst release to achieve desired MIC. The

same can be inferred from the pareto chart, half normal plot and main effect plots for Q3 (Fig. 10A and Fig. 11A) respectively. The reason may be the higher hardness which increased the bonding strength of the tablet which would have decreased the porosity and increased the tortuosity factor of the matrix which is responsible for its negative impact on drug release.

Moreover, the initial higher release was observed in all batches (Table 24). Looking deeply inside, it can be anticipated that in the beginning, drug close to surface of matrix might be released before the surrounding polymer reached the polymer disentanglement concentration. In addition, for water soluble drugs diffusional driving force would be highest and mean dissolution rates close to the mean water infiltration rates [60]. Also for high viscosity polymer like Polyox WSR 303, it would take a longer time to form a gel layer which provides enough time for initial burst release. Similar mechanisms have been reported in literature for initial high release of water soluble drug from matrix monolithic system [61]. Now interestingly, interaction effect was also observed between amount of Polyox WSR 303 and amount of EC (Table 25, Fig. 10 A). The same can be inferred from interaction plot Fig. 12A.

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

$$Q3 = 28.39 - 0.49A - 3.05B - 0.39C + 0.10AB - 0004BC + 0.79AC \quad (6)$$

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

$$Q3 = 28.39 - 3.05B + 0.79AC \quad (7)$$

Table 24: Matrix of the experiments for 2^3 full factorial design and results for the measured responses.

ES[a]	Amount of Polyox (mg/tab)	Hardness (Kp)	Amount of ethyl cellulose (mg/tab)	Q3 (hr)[b]	Q4 (hr)[b]	Q6 (hr)[b]	Q10 (hr)[b]
8	-1	-1	-1	33.16±1.2	51.79±2.2	82.42±1.9	96.50±1.5
3	1	-1	-1	30.50±0.9	44.90±3.0	78.90±2.2	96.80±0.9
5	-1	1	-1	26.97±1.3	44.20±2.9	66.15±1.1	97.90±0.8
4	1	1	-1	24.50±1.6	33.95±2.1	63.75±1.6	98.90±0.9
6	-1	-1	1	30.90±0.8	49.50±2.6	80.10±1.5	98.20±0.8
2	1	-1	1	31.20±1.6	48.90±2.3	82.20±1.2	97.80±1.2
1	-1	1	1	24.48±0.9	41.06±1.5	58.72±2.0	96.20±1.3
7	1	1	1	25.40±1.3	35.90±2.0	62.62±2.5	95.80±0.5

a Experimental sequence

b Mean±SD (n=6)

Table 25: ANOVA Results (*p*-values): Effect of the variables on Q3, Q4, Q6 and Q10.

Factors	Q3		Q4		Q6		Q10	
	Coefficient	*p* value (Prob >F)	Coefficient	*p* value (Prob >F)	Coefficient	*p* value (Prob >F)	Coefficient	*p* value (Prob >F)
Intercept	28.39	0.0313*	43.78	0.0466*	+71.86	0.0171*	+97.26	0.1601
A	-0.49	0.0697	-2.86	0.0333*	0.010	0.9254	+0.062	0.6051
B	-3.05	0.0112*	-5.00	0.0191*	-9.05	0.0060*	-0.063	0.6051
C	-0.39	0.0864	0.065	0.7397	-0.95	0.0570	-0.26	0.2048
AB	+0.10	0.3107	-0.99	0.0957	+0.37	0.1457	+0.088	0.5000
AC	+0.79	0.0430*	1.42	0.0669	+1.49	0.0363*	-0.26	0.2048
BC	-0.004	0.9557	-0.36	0.2498	-1.19	0.0453*	-0.94	0.0592

Regression coefficients are in coded value

* Statistically significant ($p < 0.05$)

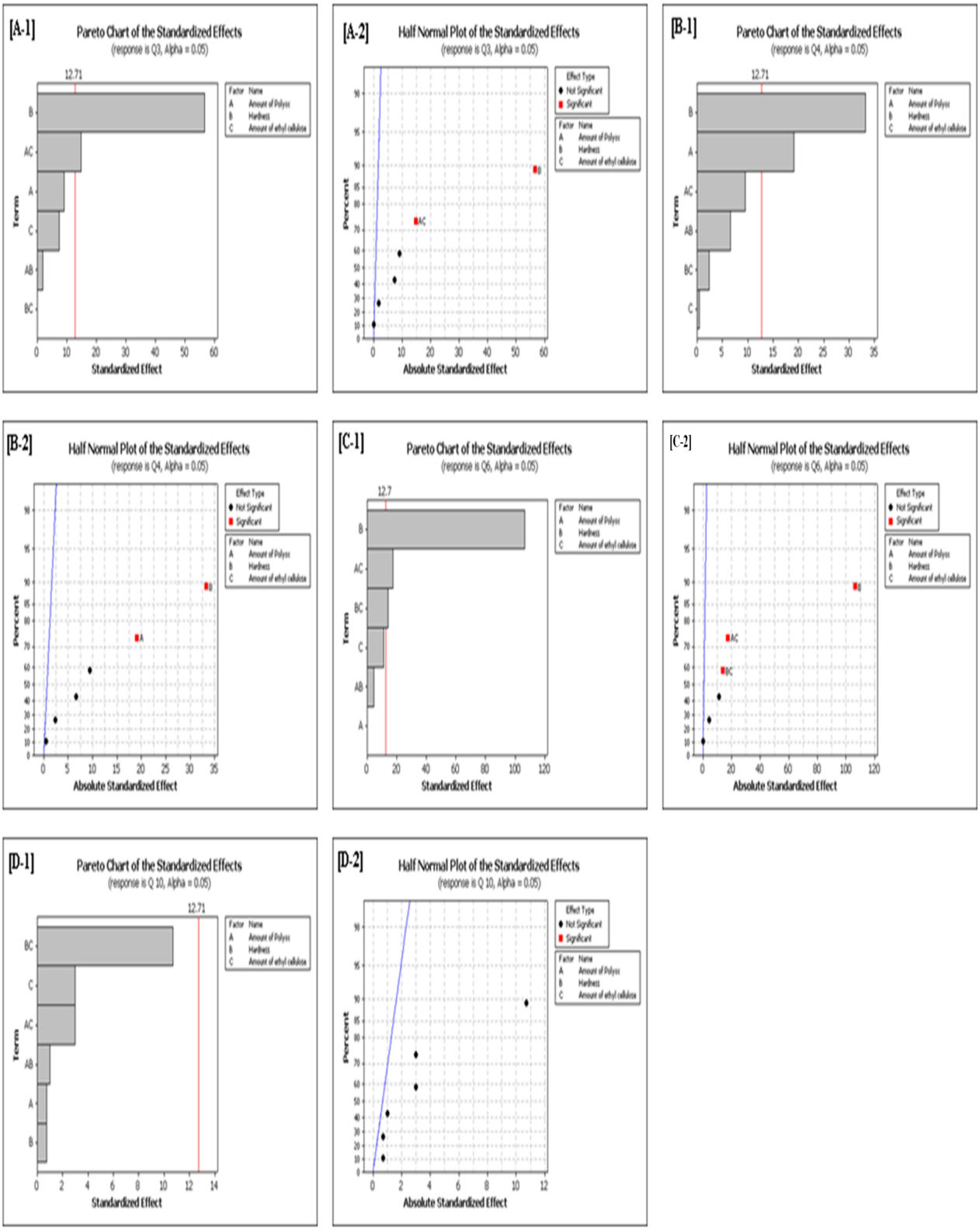


Fig. 10: Pareto chart and half normal plot of the standardized effects for responses (A) Q3, (B) Q4, (C) Q6 and (D) Q10

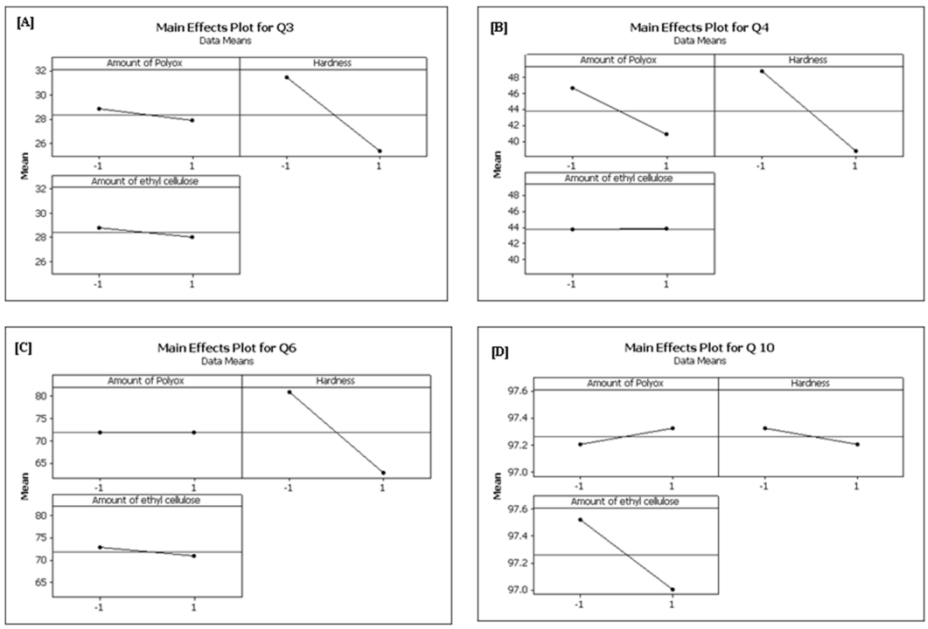


Fig.11: Main effects plot for (A) Q3, (B) Q4, (C) Q6 and (D) Q 10 as a function of amount of Polyox WSR 303, hardness and amount of ethyl cellulose.

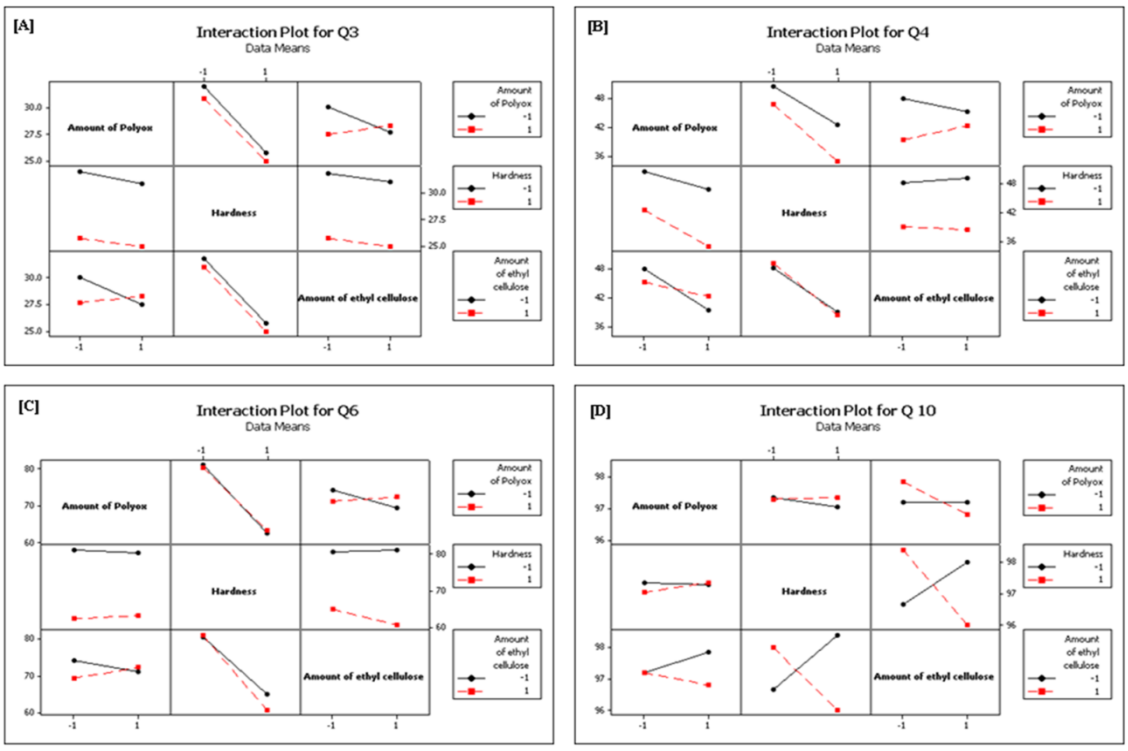


Fig.12: Interaction profile of amount of Polyox WSR 303, hardness and amount of ethyl cellulose on (A) Q3, (B) Q4, (C) Q6 and (D) Q 10.

5.1.4.7.2. Q4: Percent drug released in 2 hr in pH 6.8 phosphate buffer

From Table 25, Fig. 10B and Fig. 11B, it can be concluded that amount of Polyox WSR 303 and hardness were the most influencing factor affecting Q4 negatively (negative co-efficient; Table 5) to maintain the sustain release. The reason for factor A to retard drug release might be due to greater chain entanglement produced by high viscosity polymers, ultimately resulting in thicker gel layer after hydration of it. Furthermore, on formation of gel by high viscosity polymers, it is difficult for longer chains to dissolve easily since high energy is required for pulling them off the matrix. Similar mechanism have been proposed for high viscosity polymers for sustained drug release by some researchers [62, 63]. Thus, the longer diffusional path length created by gel layer resulted in the decreased effective diffusion of the drug and therefore a reduction in the drug release rate. The mechanism of hardness for decreasing drug release has been already discussed.

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

$$Q4 = 43.78-2.86A-5.00B+0.06C-0.99AB-0.36BC+1.42AC \quad (8)$$

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

$$Q4 = 43.78-2.86A-5.00B \quad (9)$$

5.1.4.7.3. Q6: Percent drug released in 4 hr in pH 6.8 phosphate buffer

As shown in Fig. 10C, 11C and Table 25, it can be concluded that hardness was the most influencing factor affecting Q6 negatively. The batches (e.g. ES 5,4 and1) in which hardness was at higher level could sustain drug release in desired constraints. On the contrary, batches (e.g. ES 8, 3 and 6) in which hardness was at low level; release was faster. Interestingly, interaction effect was observed between AC and BC (Table 25) on percent drug release at Q6 time point. The same can be inferred from pareto chart, half normal probability ploy (Fig. 10C) and p value of ANOVA table (Table 25). The results obtained surprisingly depict that there is no pronounced decrease in drug release on increasing binder EC concentration (Table 24 and Table 25) similar to results of Q4. The reason might be EC was added as dry binder in the premix. Then IPA was added to granulate the mass. In case of EC it was hypothesized that it dissolved on addition on IPA and it exhibits coating like effect. Moreover, addition of IPA was stopped on achieving end point in granulation. Thus it is

hypothesized that quantity of IPA was not sufficient to dissolve dry binder in the premix to exhibit more hydrophobic coating like effect and as a result much binder remained in the dry state. As a result, there might not be significant difference in providing hydrophobic interaction as compared to lower binder concentrations. Secondary reason assumed may be high solubility of the drug and drug loading. Hence, due to early achieving end point in granulation with less consumption of IPA, together with high solubility of drug there is not much significant decrease in drug release on increasing binder concentration. The exact mechanism yet needs to be investigated.

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

$$Q\ 6 = 71.86+0.01A-9.05B-0.95C+0.36AB-1.19BC+1.49AC \quad (10)$$

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

$$Q\ 6 = 71.86-9.05B+1.49AC-1.19BC \quad (11)$$

5.1.4.7.4. Q 10: Percent drug released in 8 hr in pH 6.8 phosphate buffer

For Q 10, more than 90% drug release is desired to ensure complete drug release from developed formulation. As highlighted in Fig. 10D and p value from ANOVA table (Table 25), it is clearly stipulated that none factor or interaction had influenced on percent drug release at Q10. All the batches showed more than 90% drug release at Q10. The reason may be due to high solubility of drug and drug loading. Another factor is threshold level of retardation of drug release rate by polymer as drug release does not result solely from polymer erosion, but also on drug diffusion through the hydrated polymer layers. Thus it was also observed that increased in polymer concentration changed the release pattern but could not prolong the release due to above discussed mechanism.

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

$$Q\ 10 = 97.26+0.062A-0.063B-0.26C+0.088AB-0.26AC-0.94BC \quad (12)$$

5.1.4.7.5. Model fitting and statistics of the measured responses

Here, higher values of r^2 for all dependent variables were found which statistically signify a good fit. Additionally, Adj-r^2 and Pred-r^2 values were also in reasonable

agreement signifying good model fit (Table 26). Further model showed the adequate precision value greater than 4, indicating adequate model discrimination [58]. A model fit value of $Q^2 > 0.5$ is considered as fairly good and value of $Q^2 > 0.9$ is generally taken as excellent [59]. Q^2 values for all the measured responses were good signifying good model fit. The residual plots viz., normal probability plot of residuals, residual vs fit, residual vs order and histogram of residuals for Q3, Q4, Q6 and Q 10 are depicted in Fig. 13. 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.13, there exists no evidence of the error terms to be correlated with each other.

Table 26: ANOVA results showing the effect of independent variables on the measured responses.

Measured response	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
Q3	82.75	6	13.79	596.75	0.0313	1.48	0.9997	0.9980	0.9821	61.037	0.9821
Q4	290.47	6	48.41	268.95	0.0466	11.52	0.9994	0.9957	0.9604	44.953	0.9603
Q6	629.24	6	115.37	1996.09	0.0171	3.70	0.9999	0.9994	0.9947	105.385	0.9946
Q10	8.26	6	1.38	22.47	0.1601	3.92	0.9926	0.9485	0.5288	12.635	0.5254

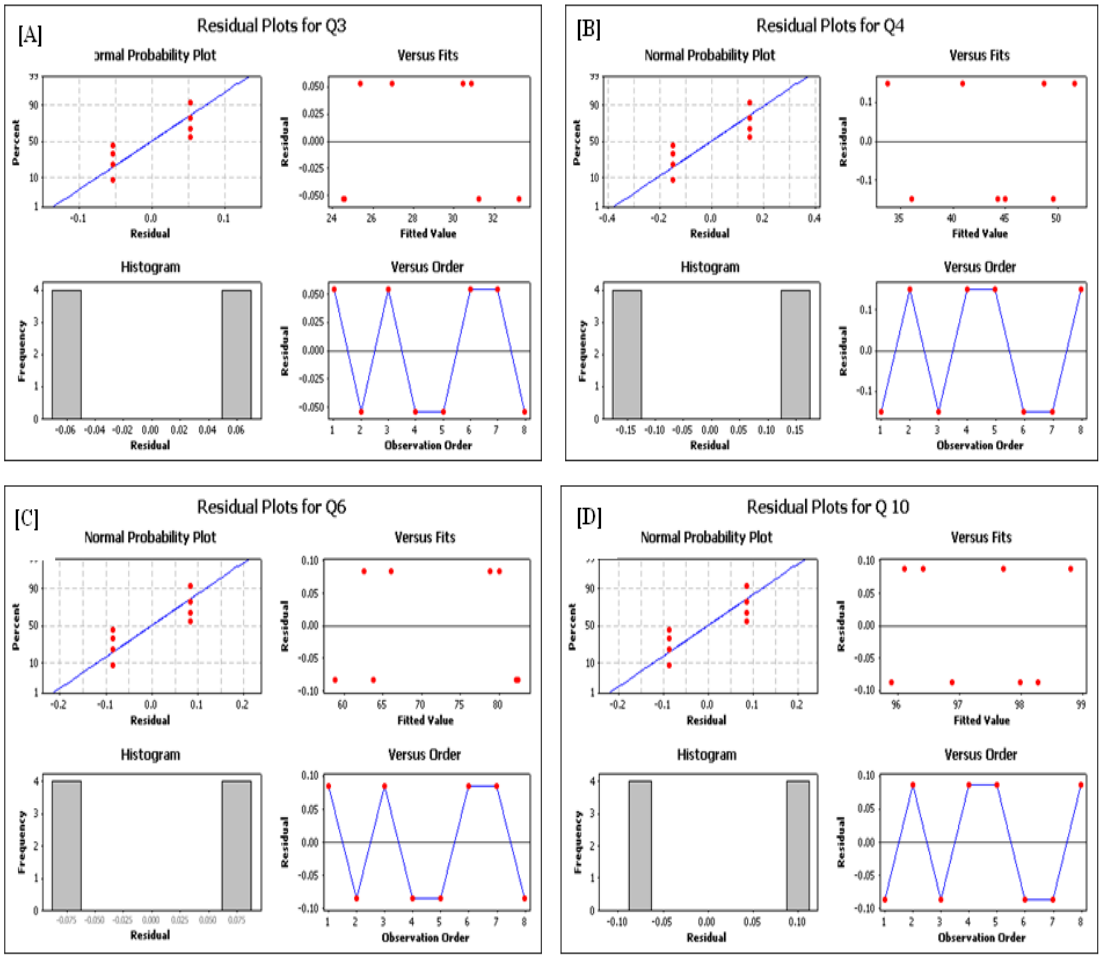


Fig. 13: Residual plots for (A) Q3, (B) Q4, (C) Q6 and (D) Q10.

5.1.4.7.6. Evaluation of model using cross-validation

The reliability of the model was assessed by conducting five experiments by varying the formulation variables at values other than that of the model. The experimental and predicted values for each response are shown in Table 27. Bias or percent relative error between predicted and experimental values for each response was calculated by the following Eq. (13).

$$\text{Bias} = \left[\frac{\text{Predicted value} - \text{Experimental value}}{\text{Predicted value}} \right] \qquad (13)$$

Results from Table 27 reveal 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 satisfactorily the influence of the chosen formulation variables on the responses under study.

Table 27: Comparison of responses between predicted and values for the cross validation set

Responses	Test	Factors/levels			Experimental values	Predicted values	Bias%
		X1	X2	X3			
Q3	1	−1	-0.6	-0.6	28.55	30.69	6.99
	2	-0.6	0	0.4	30.55	28.20	-8.33
	3	-0.4	0.6	0	27.12	26.56	-2.11
	4	0	-0.4	0.6	31.35	29.61	-5.88
	5	0.5	0.5	-0.5	27.50	26.67	-3.12
Q4	1	−1	-0.6	-0.6	50.80	49.64	-2.34
	2	-0.6	0	0.4	46.90	45.5	-3.08
	3	-0.4	0.6	0	40.10	41.92	4.34
	4	0	-0.4	0.6	44.26	45.78	3.32
	5	0.5	0.5	-0.5	41.55	39.85	-4.27
Q6	1	−1	-0.6	-0.6	75.10	77.76	3.42
	2	-0.6	0	0.4	74.10	71.50	-3.64
	3	-0.4	0.6	0	68.23	66.43	-2.71
	4	0	-0.4	0.6	71.90	75.77	5.11
	5	0.5	0.5	-0.5	65.20	67.26	3.06
Q 10	1	−1	-0.6	-0.6	95.50	96.95	1.50
	2	-0.6	0	0.4	98.66	97.18	-1.52
	3	-0.4	0.6	0	96.20	97.17	1.00
	4	0	-0.4	0.6	97.55	97.35	-0.21
	5	0.5	0.5	-0.5	95.66	97.71	2.10

5.1.4.8. Optimization using desirability function

The desirability function is an excellent tool to merge multicriteria responses in one single criterion measurement. The information obtained from it can be useful for predicting optimum levels of individual variables. If the value of the response is on target or is at optimum, its desirability value was allocated as 1 and for totally unacceptable value its desirability was given as 0. The individual desirability for each response was calculated [58] using the approaches discussed below.

Q3 was desired to be the maximum so as to achieve initial burst release of INH. But here target was specified for Q3 as it was anticipated that more of the initial release might not prolong drug release upto 8 hr. Desirability *d1* for response Q3 was calculated by Eq. 14:

$$d1 = \left[\frac{Yi - Ymin}{Ytarget - Ymin} \right] \quad (14)$$

Yi is the experimental result, and *Ymin* and *Ymax* represent the minimum and maximum possible values. *Ymax*, *Ymin* and *Ytarget* for this response were 33.16, 24.48 and 26.0 percent drug release respectively.

For Q4 and Q6 there were no specific requirements for either obtaining maximum or minimum value. Q4 and Q6 response justifies that the drug releases in sustain manner from the dosage form. For Q4 and Q6; the formulations having percentage release within the constraint range selected (Table 24) was 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. 15:

$d2,d3 = 0$ for $Yi < Y\,min$

$d2,d3 = 1$ for $Ymin < Yi < Ymax$

$d2,d3 = 0$ for $Yi > Ymax$ (15)

However for Q10 more than 90% drug should be release to ascertain complete release from the dosage forms. *Ymax* and *Ymin* for this response were 98.9 and 95.8 percent drug release respectively. Thus *d4* it was calculated by formula shown in Eq. 16.

$$d4 = \left[\frac{Yi - Ymin}{Ymax - Ymin} \right] \quad (16)$$

The overall desirability was calculated from the individual values by using the following Eq. 17.

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} \quad (17)$$

Desirability function was calculated for percent drug release at Q3, Q4, Q6 and Q10 time. Based on the composite desirability data and overlay contour plots (Fig. 14), ES 5 batch was identified as the optimum batch having desirability of 0.91. Composite desirability found out for optimized batch with the help of Minitab software was 0.90. The weight and importance was allotted 1 for each response respectively.

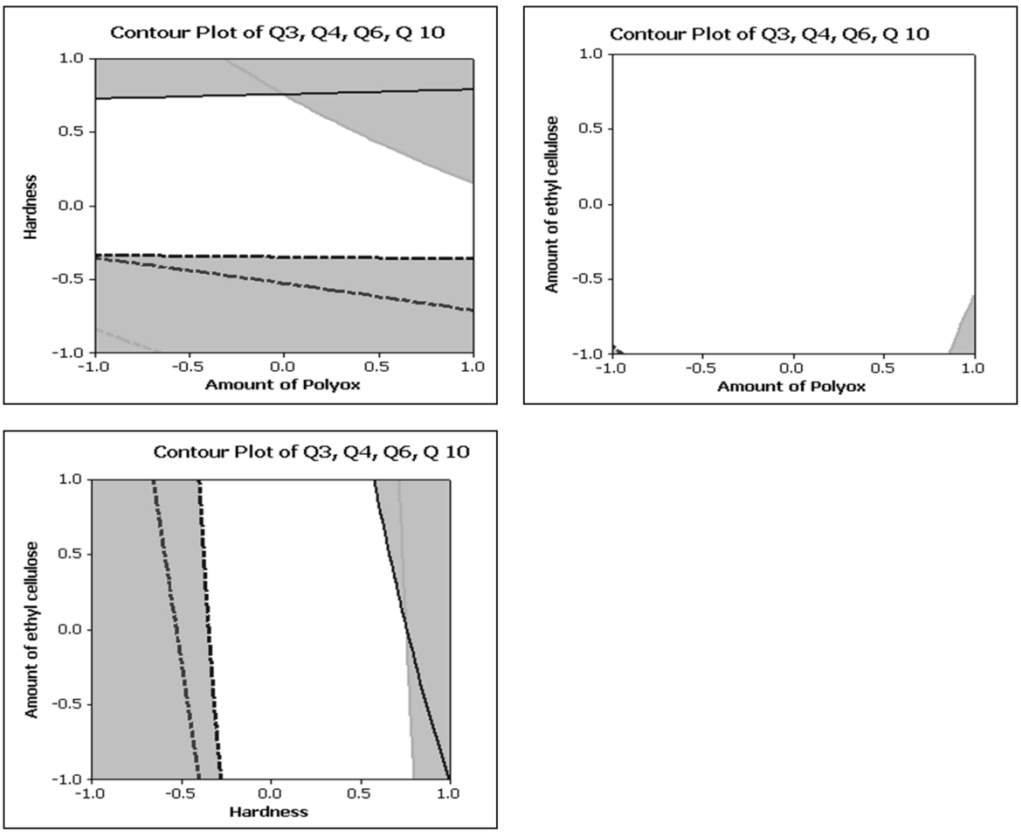


Fig. 14: Overlaid contour plots of Q3, Q4, Q6 and Q10 as a function of amount of Polyox WSR 303, hardness and amount of EC.

5.1.4.9. Curve fitting and release mechanism

Values of adjusted r^2, AIC and MSC value are presented in Table 28**.** The drug release data of the optimized batch ES 5 shows a good fit to the Korsmeyer–Peppas power law equation which can be confirmed by comparing the values of adjusted r^2 with that of the other models. The values of release exponent (n) determined for the optimized formulation batch ES 5 was found to be 0.592 suggesting the probable release by anomalous transport [36]. The lowest AIC value; 13.3878, of optimized batch ES 5 indicates that Korsemeyer–Peppas power law was the best fit model in describing the dissolution behavior. Similarly, the highest MSC, 5.4208, of the optimized batch ES 5 indicates the same.

The value of constants k1 (26.575) and k2 (2.966) of Peppas –Sahlin model are displayed in Table 28. k1 denotes relative contribution of drug diffusion to drug release and k2 denotes relative contribution of polymer relaxation to drug release.

From Table 28, it is clearly stipulated that diffusion is the predominant mechanism for drug release.

Table 28: 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 5	r^2	0.7746	0.9819	0.9806	0.9845	0.9833	0.8969	0.9779	0.9973	0.9953
	AIC	35.0412	22.4381	22.7874	21.6436	22.5971	31.1288	24.0023	13.3878	16.2368
	MSC	1.0899	3.6105	3.5407	3.7694	3.5787	1.8724	3.2977	5.4206	4.8508
									28.813 (k)	26.575 (k1)
									0.592 (n)	2.966 (k2)

5.1.4.10. Effects of granule size

The granules of optimized batch ES 5 were subjected to downsizing in two particle sizes range viz. ASTM 18/24#, and 24/30# to investigate its effects on percent drug release. Results revealed that there is no statistically significant difference in drug release for two different sizes of granules ($p>0.05$). The reason may be high water solubility of the drug together with its high percent drug loading. Probably, the high diffusion co-efficient of highly water soluble drug may have quash the effect of granule size. Hence, risk and criticality of this failure mode is low. The comparative graph of two different granule size is depicted in Fig. 15.

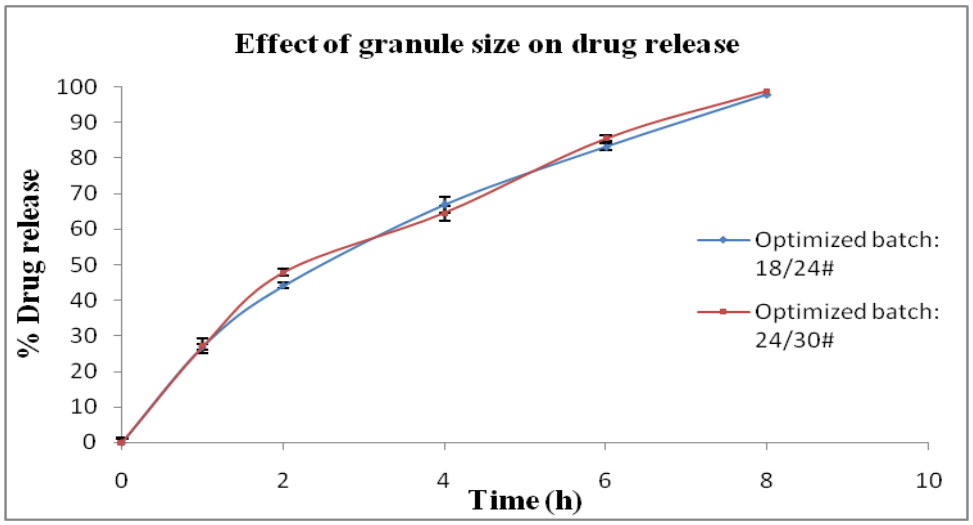
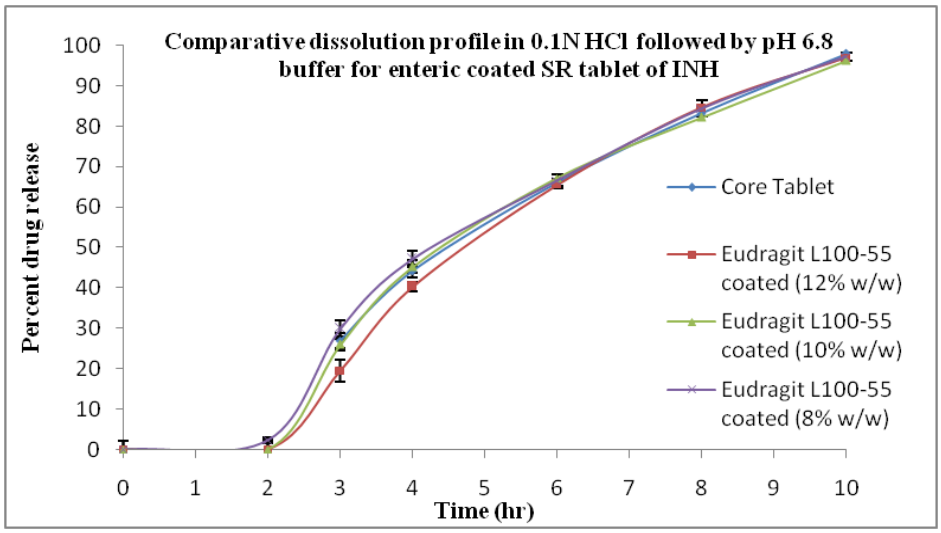


Fig. 15: Effect of granule sizes of optimized batch on *in vitro* percent drug release.

5.1.4.11. 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 10% 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 tablet in pH 6.8 phosphate buffer (Fig. 16). Weight gain of 8% w/w provided similar dissolution profile as that of core tablet in phosphate buffer pH 6.8 but simultaneously drug release of 2.3% (mean value) was obtained in acidic medium. On the contrary, 12% w/w provided zero percentage drug release in acidic medium but slow down dissolution profile of the core tablet during initial hours in pH 6.8 phosphate buffer (Fig. 16).



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

Fig.16: Dissolution of enteric coated SR tablet of INH in 0.1N HCL followed by pH 6.8 phosphate buffer.

5.1.4.12. Capability analysis

The results of normal probability plots for detecting normality of distribution are depicted in Table 29 for Q3, Q4, Q6 and Q10 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 Q3, Q4, Q6 and Q 10 are displayed in Table 29. For a process to be capable to produce batches within specifications, all the indices value should be above 1.33 [42, 45]. From the results of Table 29, it can be inferred that all the indices value were 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 Q3, Q4, Q6 and Q10.

For Q4, Cp is 2.10, which indicates that the specification spread is 2.10 times greater than the 3-σ spread in the process. Moreover Cp (2.10) and Cpk (2.05) are very close to one another, revealing that the process is centered. Regarding overall capability, Pp value is 2.13 indicating 2.13 times greater than the 3-σ spread in the process. Also Pp (2.13), Ppk (2.07) and Cpm (2.12) 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. For Q3 and Q6, Cp and Cpk are not as close to each other as compare to Q3 signifying the process is slight deviating from the center (Table 29). Nevertheless, all the indices of within and overall were above 1.33 (Table 29) and results were within desired constraints (Table 23) signifying process passes capability analysis at 3-σ standard deviation process spread. Regarding Q10, there is disparity between Cp (3.25) and Cpk (1.41), which is due to more than 95% drug release in all the batches which was desired. The same can be inferred from CPL (5.10) value. Similar conclusions can be drawn for overall indices. Furthermore, all the indices were above 1.33 (Table 29) revealing process passes capability analysis at 3- σ standard deviation. Fig. 17 portrays the pictorial representation of capability analysis for Q3, Q4, Q6 and Q10.

Table 29: Summary of the various capability indices

Variable	Potential within capability				Overall capability				
	Cp	Cpk	CPL	CPU	Pp	Ppk	PPL	PPU	Cpm
Q3	1.99	1.43	2.55	1.43	2.13	1.53	2.74	1.53	1.65
Q4	2.10	2.05	2.05	2.16	2.13	2.07	2.07	2.18	2.12
Q6	2.92	2.41	2.41	3.43	3.17	2.61	2.61	3.72	2.51
Q10	3.25	1.41	5.10	1.41	3.42	1.48	5.36	1.48	1.35
Normal Probability test results at 5% significance level									
	AD* value	p value	RJ** value	p value	KS*** value	p value			
Q3	0.381	0.379	0.988	>0.100	0.123	>0.150			
Q4	0.324	0.510	0.990	>0.100	0.112	>0.150			
Q6	0.435	0.280	0.985	>0.100	0.137	>0.150			
Q10	0.541	0.152	0.986	>0.100	0.131	>0.150			

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

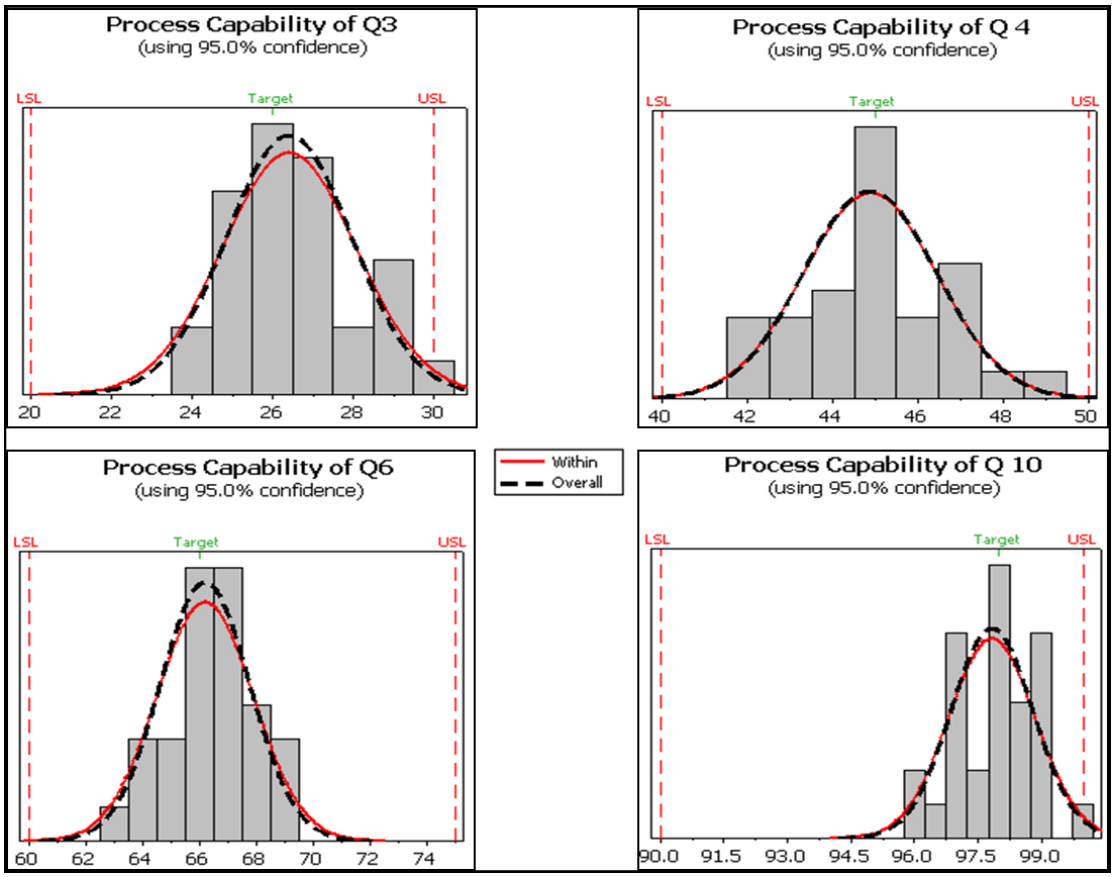


Fig. 17: Pictorial representation of capability analysis for Q3, Q4, Q6 and Q10.

5.1.4.13. Packaging and stability studies

The optimized formulation ES 5 showed insignificant change under the conditions of storage for parameters like appearance, drug content, gastric resistance and *in vitro* drug release. The similarity factor (f2) [28] was employed for comparison of

dissolution profiles on each time point. It ranged from 83 to 92. Thus the data suggested that the formulation was stable for under the packaging material selected revealing that it risks it under control and low.

5.1.4.14. Risk mitigation and control strategy

2^3 full factorial design was employed to examine the multidimensional interaction of input variables of the core tablet which were ranked as high risk in the initial risk assessment for establishment of a design space. The acceptable region within which a quality of the product can be constructed is called as design space. [21, 51]. The risk mitigation and control strategy is fused outline of how quality is established based on current process and existing product knowledge.

For factor C, it can be inferred from main effect plots (Fig. 11A–D), overlay contour plots (Fig. 14) and p value from ANOVA (Table 25) that it did not significantly affected any of the dependant variables (p value > 0.05) as main effect but showed interaction effects with other factors on some of the responses (Fig. 12A-D). Using software, we modulated the range of C by changing its setting level and observed the change in overlay contour plot of A vs B.

The range of C where region of A vs B in overlay contour plot was found to be maximum was selected as range of C. We found range of C of -0.3 to 0.6 in coded units as optimum range and thus we decided to use EC in that range. The risk with operating in this range is low. The risk mitigation strategy is to monitor the dissolution within desired constraints range.

From the ANOVA table and p value (Table 25), overlay contour plots (Fig. 14) and main effect plots (Fig. 11A–D) it is clearly observed that factor A have major impact on percent drug release at Q3 and Q4 and B on Q3, Q4 and Q6. Thus there is an optimum range for A and B where you can get the desired drug release in the set constraint range which is specified in overlay contour plot (Fig. 14) of B and C vs. all four responses. Working in this zone, risk is low as all the responses will ascertain to be in the constraint range. The risk mitigation strategy for the same is to monitor percent drug release at Q3, Q4, Q6 and Q10 are in the constraint range.

The enteric coating was also in the high risk category which was optimized as discussed in section 5.1.4.11. Regarding moderate RPN failure modes, granule size and packaging were discussed in sections 5.1.4.10. and 5.1.4.13. Fig.18 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 30 and Table 31. 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.

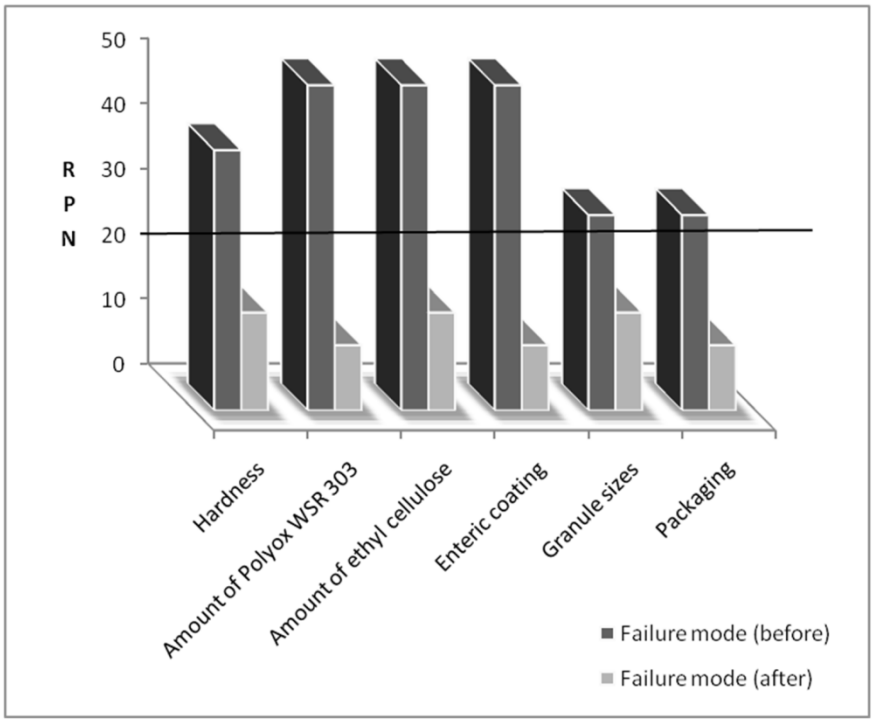


Fig. 18: FMEA analysis of INH site specific sustain release tablet depicting RPN number of failure mode before and after implementation of control strategy.

Table 30: Final and updated risk based matrix analysis for identification of impact of formulation ingredients on drug product attributes.

DP CQAs*	Polymer (Polyox WSR303)	Binder	Filler	Aerosil 200	Magnesium stearate
Hardness	Low	Low	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 31: Final and updated based matrix analysis for identification of impact of unit operations on drug product attributes.

DP CQAs*	Sizing	Blending	Granulation	Compression	Granule sizing	Enteric Coating
Hardness	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

5.1.5. Conclusion

There is no ambiguity that several initiatives are undertaken worldwide to circumvent development hiccups of anti-TB formulations. The formulation technology used here is simple, easily scalable and adopted in industries. Hence, it endows to be of greater interest especially in under developed or developing countries to epitomize the objectives like cost-effectiveness, feasibility and save resources. 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 of QbD, 2^3 full factorial design was employed for evaluating the failure modes with high RPN number of core tablet and defining the relationships between input variables and quality traits desired. The optimized formulation exhibited percent release at Q3 of 26.97%, Q4 of 44.20%, Q6 of 66.15%, Q10 of 97.9% and gastric resistance less than 10%. Finally, the design space was established and control strategy was developed to mitigate the risk in future. The RPN of updated risk assessment represents that all the failure modes of FMEA analysis were in low risk category (Fig. 18). Finally, capability indices were performed on five reproducibility batches and results revealed that all indices were above 1.33 indicating process was significantly under control.

Thus the shift in exemplar from traditional approach to QbD approach can provide incisive insight for building quality within the product. Hence, the developed formulation may provide prudently a better substitute for conventional tablet 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.

5.1.6. References

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