Research Article

Formulation and Evaluation of a Novel Capsule-in-a-Capsule Technology of Anti-tubercular Drugs

Mohd Abdul Hadi[1*], Sayeeda Tabassum[2], Tayyaba Mahtab[2], Rahamat Unissa[3], Dr. A. Srinivasa Rao[4]

[1*]Department of Pharmaceutics, Bhaskar Pharmacy College (JB Group of Educational Institutions), Yenkapally (V), Moinabad (M), R.R.District, Hyderabad, Telangana, India.

[2]Department of Pharmaceutical Analysis, Bhaskar Pharmacy College (JB Group of Educational Institutions), Yenkapally (V), Moinabad (M), R.R.District, Hyderabad, Telangana, India.

[3]Department of Pharmaceutics, College of Pharmacy, University of Hail, Saudi Arabia.

[4]Department of Pharmacy Practice, Bhaskar Pharmacy College (JB Group of Educational Institutions), Yenkapally (V), Moinabad (M), R.R.District, Hyderabad, Telangana, India.

***Corresponding author's E-mail:** hadi.lcp@gmail.com

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ABSTRACT

The present investigation aims to develop a novel capsule-in-a-capsule technology using multiple unit mini-tablets for targeting and sustaining the release of rifampicin and isoniazid in stomach and intestine respectively. Before developing the batches, drugs and polymers were checked for compatibility studies. For preparing the formulation, rifampicin was developed as liquid dispersions and floating mini-tablets using various solvent mixtures and hydrophilic polymers respectively. Whereas, isoniazid was developed as intestinal targeted mini-tablets using pH-dependent polymers. Moreover, the capsule-in-a-capsule formulation was developed by first filling five isoniazid mini-tablets into a smaller sized capsule (i.e. size "3") and then smaller mini-tablets-filled capsule of isoniazid and ten rifampicin mini-tablets into a bigger sized capsule (i.e. size "0"). FTIR and DSC studies confirm that there was no interaction between drug and polymers. From the separate in-vitro dissolution studies, it was found that rifampicin floating mini-tablets containing 30% concentration of HPMCK-4M and HPMC-K100M polymers in 1:4 ratio and intestinal targeted isoniazid mini-tablets containing 50% concentration of eudragit-S100 polymer were considered as the most optimized batches. Whereas, capsule-in-a-capsule formulation released 96.92±1.14 % of rifampicin at the end of 4 hours and 4.17±1.68 %, 99.06±1.88 % of isoniazid at the end of 2 and 6 hours respectively. This formulation was also found to be stable as per the ICH guidelines. The developed capsule-in-a-capsule formulations have successfully released rifampicin and isoniazid in the pH of stomach and small intestine respectively as observed from the in-vitro results.

Keywords: Rifampicin; Isoniazid; Capsule-in-a-capsule technology; Liquid dispersions; Floating mini-tablets; Intestinal targeted mini-tablets.

INTRODUCTION

Tuberculosis is a deadly and common infectious disease which is caused by mycobacterium, mainly mycobacterium tuberculosis.[1] Since past forty years, rifampicin and isoniazid has been majorly used in tuberculosis therapy.[2,3] It is because isoniazid is a first-line anti-tubercular drug and it acts by inhibiting the mycolic acid synthesis in the mycobacterium cell wall. Isoniazid is never used alone to treat active tuberculosis because of its quick resistance to the body. Whereas, rifampicin is a novel and only anti-tubercular drug which has the unique ability to kill dormant tubercular bacilli. It acts by inhibiting DNA-dependent RNA polymerase in the bacterial cells by binding to its beta-subunit, thereby preventing RNA transcription and subsequent proteins translation.[4]

Isoniazid and rifampicin are widely prescribed as combination product for tuberculosis treatment. But since past years, two critical problems have been observed from such combination products of isoniazid and rifampicin. That includes 1) The impaired and varying bioavailability of rifampicin from combination formulation with isoniazid and 2) Poor rifampicin stability containing combination formulation with Isoniazid.[5-7] The possible reason for such problem is that the rifampicin interacts with isoniazid in the acidic media of stomach to form inactive 3-formyl rifamycin isonicotinyl hydrazone. Thus, the use of substandard combination formulations ultimately results in the emergence of drug resistant tuberculosis and hence treatment failure.[8] Moreover, it has also been found that rifampicin is highly soluble between 1-2 pH and well absorbed from the stomach. Whereas, isoniazid is well absorbed from all the three sections of small intestine (i.e. duodenum, jejunum and ileum).[9] Thus, there is a necessity to modify the combination formulation in such a way that rifampicin and isoniazid are not released simultaneously in the stomach.

Thus, the innovative thinking has transformed towards the novel concept of capsule-in-a-capsule technology of Anti-tubercular drugs with improved functionality. It is because capsule-in-a-capsule drug delivery systems are ideally suited for combination or dual release products. They can be used to combine incompatible active pharmaceutical ingredients and to deliver compounds to two different regions of gastro-intestinal tract. The best advantage of this novel technology is that it offers several

therapeutic possibilities and a broad range of design and formulation options. This single oral dosage delivery system is developed by inserting a smaller pre-filled capsule into a larger liquid-filled capsule. The inner capsule of this unique and novel drug delivery system can be filled with liquid, semi-solid, powder, mini-tablets or pellets, while the outer capsule can be filled with liquid or semi-solid or solid formulation.[10,11]

The current research aims to develop capsule-in-a-capsule formulation by filling inner capsule with intestinal targeted isoniazid mini-tablets and the outer capsule with either rifampicin liquid dispersion or floating mini-tablets. As mini-tablets can be successfully used as multiple unit modified release systems (delayed colon release, controlled release, pulsatile and bi-modal release and gastro-retentive systems) thereby providing improved drug bioavailability compared with single unit dosage forms. Moreover, these multiple unit mini-tablets are having all the manufacturing and economic advantages of a single unit larger tablet, and still the problems such as chances of dose dumping, systemic toxicity, and changes in formulation behavior and drug release profile due to unit to unit variation can be avoided. Also, these mini-tablets are very simple and easier to prepare by using direct compression technique thereby involving very less steps using simpler equipments thus saving the time and cost. Various other mini-tablet benefits include excellent size, weight uniformity and regular shapes.[12-14] Whereas, the advantages of Liquid-filled-capsule technology is well established for formulation and manufacture.[15-17]

Hence, the objective of the present research was to develop a novel capsule-in-a-capsule formulation which during *in-vitro* dissolution testing sustains the release of rifampicin for a period of atleast 4 hours within the pH media of stomach and targets the release of isoniazid within pH medias of small intestine.

MATERIALS AND METHODS

Rifampicin, isoniazid, polyvinyl pyrrolidone-K30, beta Hydroxy Toluene and ethyl alcohol were purchased from Yarrow chem, Mumbai, India. Eudragit® L-100 and eudragit® S-100 polymers were obtained as gift samples by Degussa India Pvt. Ltd., Mumbai, India. Sodium lauryl sulphate, polyethylene glycol-400, propylene glycol, microcrystalline cellulose and aerosil were purchased from SD. Fine Chemicals, Mumbai, India. Magnesium stearate was purchased from Himedia Chem Lab, Mumbai, India. Whereas, almost all sizes of empty HPMC capsules were obtained as gift samples from ACG Associated capsules Pvt. Ltd., Mumbai, India. Remaining all other materials used was of analytical grade.

Preformulation studies

Procedure for Fourier Transform Infrared (FTIR) spectral analysis

The compatibility for pure drugs rifampicin, isoniazid, polymers and their respective physical mixtures (in equal ratio) used in the present experimental design of liquid dispersions and mini-tablets were checked by recording of spectra using FTIR Spectrophotometer (Perkin Elmer, spectrum-100, Japan). The spectras were recorded by using 5 % of sample in potassium bromide (KBr) mixture which was made into a fine powder and was finally compressed into KBr pellets at 4000 Psi compaction pressure for 2 min. The range of scanning was 400 - 4000 cm^{-1} and the resolution was 1 cm^{-1}.[18, 19]

Procedure for Diffraction Scanning Calorimetric (DSC) studies

The DSC thermograms of pure drugs rifampicin, isoniazid, polymers and their respective physical mixtures (in equal ratio) used in the present experimental design of liquid dispersion and mini-tablets were recorded using Diffraction scanning calorimeter (DSC 60, Shimadzu, Japan). The study was carried out by taking 10 mg of accurately weighed samples which were hermetically sealed in flat bottom aluminum pans and the measurement was done at a temperature ranging between 30 °C to 350 °C at a heating rate of 10 °C/min under an atmosphere of nitrogen.[18, 19]

Procedure for pre-compression parameters

In order to study the flow properties and to maintain the weight uniformity, the prepared powder blends of mini-tablet batches were evaluated for pre-compression parameters.

Angle of repose (θ)

The Angle of repose value was determined by taking accurately weighed powder blend quantity into the funnel. The height of the funnel was adjusted so that funnel tip should touch the apex of blend. The powder blend was then allowed to flow freely through the funnel onto the surface. Thus, from the formed powder cone, height and radius were measured and the Angle of repose was calculated using the equation mentioned below.

$$\tan \theta = h/r$$

Where h and r are the height and radius of the formed powder cone respectively.[20] The average of three consecutive values was noted.

Loose Bulk density (LBD) and Tapped Bulk density (TBD)

The LBD and TBD values were determined by weighing accurately 2 gm of prepared powder blend from each mini-tablets batch which was shaken previously to break any formation of agglomerates, and was then introduced into a 10 ml measuring cylinder. By weighing initially, the measuring cylinder was then allowed to fall under its own weight onto a hard surface from 2.5 cm height at a time period of 2 sec intervals. This tapping process was continued until no further change in powder blend volume was noted and their LBD and TBD values were calculated using the equations mentioned below. [20] The average of three consecutive values was noted.

LBD= Weight of the Granules /Untapped Volume of the packing

TBD= Weight of the Granules /Tapped Volume of the packing

Hausner's ratio

Hausner's ratio is an indirect index measure for ease of powder flow. It was calculated using the equation mentioned below.[20]

$$\text{Hausner ratio} = \frac{\rho_t}{\rho_d}$$

Where, ρ_t is the tapped density and ρ_d is the bulk density. The average of three consecutive values was noted.

Carr's compressibility index

Carr's Index (%) was calculated using the equation mentioned below.[20] The average of three consecutive values was noted.

$$\text{Carr's Index (\%)} = \frac{\text{TBD - LBD}}{\text{TBD}} \times 100$$

Procedure for the preparation of rifampicin liquid dispersion (RLD)

RLD were prepared according to the formula shown in Table 1. Initially, accurate weighed quantity of rifampicin was transferred into a beaker containing propylene glycol and polyethylene glycol-400 followed by the addition of all other ingredients so as to completely dissolve the drug. The prepared liquid dispersion mixture was sonicated for 10 minutes in order to remove the entrapped air. The weight of liquid ingredients such as ethyl alcohol, distilled water, propylene glycol, polyethylene glycol-400 and beta hydroxy toluene was converted to volume from their density values and was considered accordingly.[15-17]

Table 1: Composition of rifampicin Liquid-filled capsules

Ingredients	RLD-1	RLD -2	RLD -3	RLD -4
Rifampicin	100	100	100	100
Polyvinyl pyrrolidone-K30	0.000	15	30	45
Propylene glycol	103	88	73	58
Polyethylene glycol-400	50	50	50	50
Sodium lauryl sulphate	6	6	6	6
Ethyl alcohol	35	35	35	35
Distilled Water	5	5	5	5
Beta Hydroxy Toluene	1	1	1	1
Total weight (mg)	300	300	300	300

Procedure for the preparation of floating rifampicin mini-tablets (RMT)

RMT were prepared using direct compression method as per the formula shown in Table 2. Initially, rifampicin, polymers, sodium bicarbonate, citric acid, PVP-K30 and microcrystalline cellulose were passed through the 60 mesh size sieve and after weighing according to the formulation table they were mixed. Later on, magnesium stearate and talc were separately passed through the similar sieve and after weighing they were added to the above blend and mixed thoroughly. This prepared rifampicin blend was then compressed into mini-tablets by using 3 mm round concave punches in a rotary tablet press (Rimek mini-press, RSB 4 Model, Karnavati Engineering, Ahmadabad).[12-14, 21,22]

Table 2: Composition of floating rifampicin mini-tablets

Ingredients	RMT-1	RMT -2	RMT -3	RMT -4
Rifampicin	10	10	10	10
HPMC K4M	6	4.5	3	1.5
HPMC K100M	1.5	3	4.5	6
Sodium bicarbonate	4	4	4	4
Citric acid	2	2	2	2
PVP-K30	1	1	1	1
Microcrystalline cellulose	0.25	0.25	0.25	0.25
Talc	0.125	0.125	0.125	0.125
Magnesium stearate	0.125	0.125	0.125	0.125
Total weight (mg)	25	25	25	25

Procedure for the preparation of isoniazid mini-tablets (IMT)

IMT were prepared using direct compression method as per the formula shown in Table 3. Initially, isoniazid, polymer and microcrystalline cellulose were passed through the 60 mesh size sieve and after weighing according to the formulation table they were mixed. Later on, magnesium stearate and aerosil were separately passed through the similar sieve and after weighing they were added to the above blend and mixed thoroughly. This prepared isoniazid blend was then compressed into mini-tablets by using 3 mm round concave punches in a rotary tablet press (Rimek mini-press, RSB 4 Model, Karnavati Engineering, Ahmadabad).[12-14,]

Table 3: Composition of isoniazid mini-tablets

Ingredients	IMT-1	IMT -2	IMT -3	IMT -4
Isoiniazid	10	10	10	10
Eudragit L100	5	10	--	--
Eudragit S100	--	--	5	12.5
Microcrystalline cellulose	9.5	4.5	9.5	2
Magnesium stearate	0.125	0.125	0.125	0.125
Aerosil	0.125	0.125	0.125	0.125
Total weight (mg)	25	25	25	25

Preparation of capsule-in-a-capsule formulation of rifampicin and isoniazid

In order to prepare a capsule-in-a-capsule formulation as shown in **Figure 1**, the prepared optimized IMT-4 mini-tablets equivalent to 50 mg of isoniazid (i.e. 5 mini-tablets) were filled into a smaller HPMC capsule body (i.e. size '3') and was closed with its cap. This prepared smaller IMT-4 filled capsule was then filled into a bigger HPMC capsule body (i.e. size '0') which was further filled with the optimized RMT-4 mini-tablets equivalent to 100 mg of rifampicin (i.e. 10 mini-tablets). The developed capsule-in-a-capsule formulation of rifampicin and isoniazid was then stored at room temperature until testing.[10, 11]

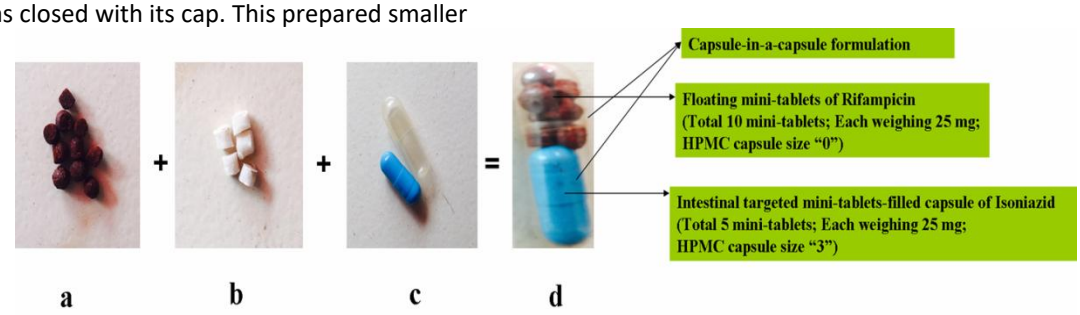

Figure 1: a) Floating mini-tablets of Rifampicin b) Intestinal targeted mini-tablets of Isoniazid c) Empty HPMC capsules (Size "0" & "3") d) Capsule in-a-capsule formulation

Evaluation methods

Visual appearance, pH and Viscosity for Liquid dispersion batches of rifampicin

Rifampicin liquid dispersion batches were evaluated for Appearance, pH and Viscosity. Appearance is considered as one of the most important parameter and it was evaluated by visual appearance of clarity. The pH was estimated by using Systronics μ pH system 361, Gujarat, India. The viscosity of the freshly prepared rifampicin liquid dispersion batches were estimated by using Brookfield viscometer and were carried out at room temperature. All the estimations were carried out in triplicate.[15-17]

Procedure for Post-compression parameters

In order to study their physicochemical properties, the prepared mini-tablet batches were evaluated for post-compression parameters.

Hardness test

The hardness of all the RMT and IMT mini-tablet batches was determined using Pfizer hardness tester by randomly taking three mini-tablets from each batch and their values were calculated**.** [20]

Friability test

The Friability test was evaluated by initially weighing ($W_{initial}$) 20 mini-tablets and then transferring them into a Veego friabilator. The friabilator was run upto 100 revolutions and operated at 25 rpm. Then the mini-tablets were weighed again (W_{final})**.** [20]

The friability percentage was determined by using the equation mentioned below.

$$\% F = \frac{W_{initial} - W_{final}}{W_{initial}} x100$$

Weight variation test

The test for weight variation was evaluated by randomly taking 20 mini-tablets from each RMT and IMT batches and then individually weighing them to check their weight variation.[20]

Uniformity of thickness

The Uniformity of mini-tablets thickness was evaluated by randomly taking 6 mini-tablets from each RMT and IMT batches and then measuring them individually using screw gauge.[20]

Drug content uniformity

The drug content uniformity for RLD batches was estimated by taking 0.3 ml of liquid dispersion equivalent to 100 mg of rifampicin in test tube and the volume was made upto 20 ml with 0.1N HCl. Whereas for RMT batches, twenty mini-tablets were randomly chosen and crushed into a mortar. Then the weighed powder containing equivalent to 100 mg of rifampicin was extracted in 20 ml of 0.1N HCl. Both the RLD and RMT samples were then filtered through a Millipore filter of 0.45 µm pore size and after suitable dilutions the rifampicin content was determined at a visible wavelength region of 473 nm using UV-Spectrophotometer. The drug content estimation was carried out in triplicate and the average value was noted.

Whereas the drug content uniformity for IMT batches was determined by randomly selecting ten mini-tablets and crushing them into a mortar. Then the weighed powder containing equivalent to 50 mg of isoniazid was extracted in 20 ml of pH 7.4 phosphate buffer. The IMT samples were then filtered through a Millipore filter of 0.45 µm pore size and after suitable dilutions the isoniazid content was determined at a wavelength of 262 nm using UV-Spectrophotometer . The drug content estimation was carried out in triplicate and the average value was noted.

***In-vitro* Buoyancy / Floating Test**

The *in-vitro* buoyancy test was determined by using floating lag time. In this method, the mini-tablets were dipped into a 100 ml beaker containing 0.1N HCL (pH 1.5). Then the time required for mini-tablet to rise to the surface and then float was determined as floating lag time and total time duration by which mini-tablet remain buoyant is called as Total Floating Time (TFT).[21,22]

***In-vitro* dissolution studies**

Separate dissolution testing evaluation was carried out in order to select the optimized RLD, RMT and IMT batches for developing the capsule-in-a-capsule formulation as per our desired criteria. Testing was performed by using USP XXIII dissolution test apparatus (paddle method). Temperature and Rotation speed were maintained at 37± 0.5 ^{o}C and 100 rpm respectively for all the batches and capsule-in-a-capsule formulation.

For RLD and RMT batches, dissolution testing was carried out in 750 ml of 1.2 pH media for a period of 4 hours. Whereas for IMT batches, in order to match the increased pH changes along the Gastro intestinal tract, four dissolution medias with pH 1.2, 6.5, 6.8 and 7.2 were used sequentially. These four media represents the stomach, proximal and distal parts of the small intestine, and terminal ileum respectively. During dissolution testing, 750 ml of 1.2 pH media was used for first two hours and was then continued with 900 ml of 6.5, 6.8 and 7.4 pH phosphate buffers for a period of 1, 2 and 1 hours respectively.

Moreover, dissolution testing evaluation was also carried out for the complete capsule-in-a-capsule formulation to assess the *in-vitro* release of both the drugs (rifampicin and isoniazid) when administered in combination. As rifampicin was formulated as floating mini-tablets and isoniazid as intestinal targeted mini-tablets. For this purpose, the dissolution study was initially carried out in 750 ml of 1.2 pH media for first two hours. After 2 hours, isoniazid mini-tablets were removed from the dissolution apparatus and transferred into another USP XXIII dissolution test apparatus containing 900 ml of pH 6.5, 6.8 and 7.2 phosphate buffers for a period of 1, 2 and 1 hours respectively. Whereas, the dissolution study of floating mini-tablets of rifampicin was carried out for another 2 hours in the same dissolution apparatus containing 750 ml of 1.2 pH media.

For all the RLD batches, 5 ml of dissolution media was withdrawn at fixed time intervals (i.e. 0, 3, 6, 9, 12, 15, 20, 30, 60, 120, 180 and 240 minutes) and was then replaced with fresh respective dissolution media. Whereas, for all the RMT, IMT batches and capsule-in-a-capsule formulation, 5 ml of dissolution media was withdrawn at fixed time intervals (i.e. 0, 15, 30, 60, 120, 180, 240, 300 and 360 min), and was then replaced with fresh respective dissolution media.

The withdrawn samples of rifampicin were analyzed at a visible wavelength region of 473 nm for 1.2 pH media and withdrawn samples of isoniazid were analyzed at 262 nm for 6.5, 6.8, 7.4 pH medias by UV-absorption spectroscopy and the percentage drug release were calculated over the sampling time intervals.

For all RLD, RMT, IMT batches and capsule-in-a-capsule formulation, the release profile was determined thrice, and average values were presented in the form of graphical representation.[23,24]

Stability studies

The stability studies for optimized capsule-in-a-capsule formulation were performed at both room temperature and accelerated stability conditions. The room temperature (RT) storage conditions were kept at 30 ±2 °C and 65 ±5% relative humidity (RH) and for accelerated stability conditions were stored at 40 ±2 °C and 75 ± 5% RH in a stability chamber. At regular time intervals of 3 and 6 months, samples were withdrawn from the stability

chamber and were checked for physical parameters such as Appearance, Weight variation, Hardness, Thickness, Friability, Drug content and *in-vitro* release profile.[25,26]

RESULTS AND DISCUSSION

Preformulation studies

FT-IR studies

In order to check drug-polymers compatibility, the spectra of pure drugs (rifampicin, isoniazid), polymers and their respective physical mixtures in RLD and RMT batches were recorded as shown in Figure 2 (FTIR). In the RLD and RMT batches, rifampicin was used as the model drug and PVP-K30, HPMC-K4M, HPMC-K100M as polymers respectively. Rifampicin has shown -OH, -NH, -C=O, C=C and -C-N stretchings due to the presence of characteristic peaks at 3427.85 cm^{-1}, 2952.87 cm^{-1}, 1718.38 cm^{-1}, 1646.88 cm^{-1} and 1377.20 cm^{-1} respectively. These are all the characteristic peaks of rifampicin. The PVP-K30 polymer spectra has shown $–CH_2$, -C-H, -C=O and -C-N stretchings due to the presence of characteristic peaks at 2947.05 cm^{-1}, 2158.18 cm^{-1}, 1705.22 cm^{-1} and 1415.48 cm^{-1} respectively. Whereas, the HPMC K4M polymer has shown –OH, -C-H and -C=O stretchings due to the presence of characteristic peaks at 3474.52 cm^{-1}, 2832.53 cm^{-1} and 1378.63 cm^{-1} respectively. The HPMC-K100M polymer has shown –OH, -C-H and -C=O stretchings due to the presence of characteristic peaks at 3479.64 cm^{-1}, 2816.57 cm^{-1} and 1386.84 cm^{-1} respectively.

Moreover in the IMT mini-tablet batches as shown in Figure 3 (FTIR), isoniazid was used as the model drug and Eudragit-L100, Eudragit-S100 as polymers. Isoniazid has shown -NH, –C=O, -C=C and C-NH stretchings due to the presence of characteristic peaks at 3110.24 cm^{-1}, 1673.38 cm^{-1}, 1505.73 cm^{-1} and 13367.40 cm^{-1} respectively. The eudragit-L100 polymer spectra has shown –OH, $-OCH_3$, $-CH_3$ and -C=O stretchings due to the presence of characteristic peaks at 3258 cm^{-1}, 2997 cm^{-1}, 2952 cm^{-1} and 1731 cm^{-1} respectively. Whereas, eudragit-S100 polymer spectra also shows similar –OH, $-OCH_3$, $-CH_3$ and –C=O stretchings due to the presence of characteristic peaks at 3225 cm^{-1}, 2998 cm^{-1}, 2953 cm^{-1} and 1727 cm^{-1} respectively.

Finally, when the physical mixture spectras of pure drugs (rifampicin and isoniazid) was recorded with their respective polymers as per the formulation tables, it was found that their respective higher spectra has also shown all the peaks of pure drugs and polymers. None of the peak was absent, as they were found to be intact. Thus, it confirms that the combination of the used drugs and polymers in liquid dispersion (RLD) and mini-tablet batches (both RMT and IMT) can be suitable for designing a capsule-in-a-capsule formulation needed for its desired therapeutic purpose.

DSC studies

The above observation of FTIR studies was further confirmed by DSC studies. The DSC thermograms of pure drugs (rifampicin, isoniazid) and the physical mixtures with their respective polymers as per formulation table are shown in Figure 2 and 3 (DSC) respectively. The DSC thermograms of pure drug rifampicin and isoniazid, corresponding to their melting point have shown sharp endothermic peaks at 191.71 °C and 170.54 °C respectively. Whereas, the DSC thermograms of PVP-K30, HPMC-K4M, HPMC-K100M, Eudragit L100 and Eudragit S100 polymers have also shown sharp endothermic peaks at 69.26 °C, 221.83 °C, 221.96 °C, 217.15 °C and 188.48 °C respectively. However, the DSC thermograms for physical mixtures of rifampicin with PVP-K30 and HPMC-K4M+HPMC-K100M and isoniazid with eudragit L100+eudragit S100 polymers have not shown any significant shift in their endothermic peaks as their peaks were found at 192.31°C, 192.89 °C and 171.12 °C respectively. Thus, the DSC thermograms results have also confirmed that the drug polymer physical mixtures used in RLD, RMT and IMT batches were free from any chemical interaction.

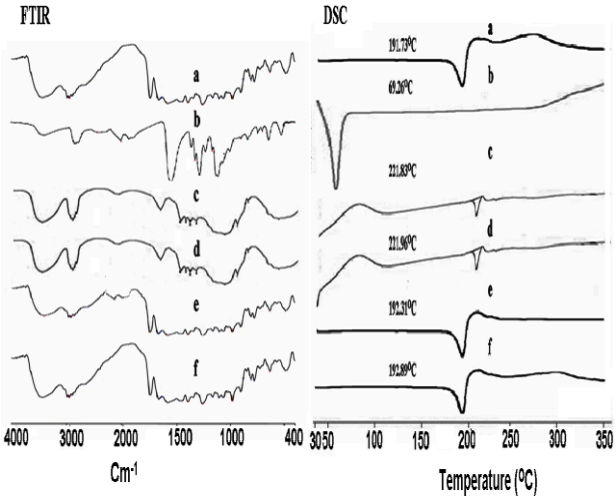

Figure 2: FT-IR and DSC spectras of a) Rifampicin b) PVP-K30 c) HPMC-K4M d) HPMC-K100M e) Physical mixture of Rifampicin+PVP-K30 f) Rifampicin+HPMC-K4M+HPMCK100M

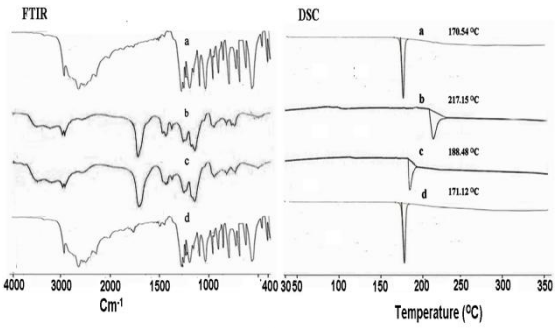

Figure 3: FT-IR and DSC spectras of a) Isoniazid b) Eudragit-L100 c) Eudragit-S100 d) Physical mixture of Isoniazid+Eudragit L100+Eudragit S100

Visual appearance, pH, Viscosity and Drug content uniformity for Liquid dispersion batches of rifampicin

The prepared RLD batches were visually tested for color and smoothness. From the visual appearance results it was found that RLD-1, RLD-2 batches were highly dark red color viscous liquids and this viscosity increased to a greater extent in RLD-3, RLD-4 batches and finally converted into a paste. Further**,** the prepared RLD batches were also evaluated for viscosity by using Brookfield viscometer and were found to range between 159.6±0.18 to 221.7±0.16. The results have clearly shown increase in viscosity of liquid dispersions as the concentration of PVP-K30 has increased. This is due to the presence of PVP-K30, a viscosity enhancing agent and also rifampicin quantity which was high in comparison to little volume of solvents. Also, the volume capacity which was remaining in size "0" HPMC capsule after getting filled with smaller size "3" capsule was only 0.3 ml. So, it was not possible to increase the quantity of solvents for diluting the drug. Hence, rifampicin converted into a paste after mixing with PVP-K30 and little quantity of solvents. Whereas, pH of all the RLD batches were in the range of 6.21±0.15 to 6.33±0.17. Thus, the pH indicates that these liquid mixtures were suitable to be filled in hard gelatin capsules as the obtained values were within the limits (i.e. 4-8) and hence the test was passed. Whereas, excellent drug content uniformity was also found in liquid dispersion batches, as their values were found to range between 98.79±0.42 to 99.42±0.52 which is more than 95%.

Evaluation of the prepared powder blends of rifampicin and isoniazid

The Angle of repose values for the powdered blend of RMT and IMT mini-tablets was found to range between 23°.55'±0.10 to 24°.77'±0.10. The LBD and TBD values were found to range between 0.511±0.01 to 0.526±0.01 and 0.579 ± 0.01 to 0.595±0.01 gm/cc respectively. The Carr's compressibility index values were found to range between 11.50±0.73 to 12.10± 0.78 %. The Hausner's ratio values were found to range between 1.13±0.00 to 1.13±0.01. As, angle of repose values were found to be less than 30º, Carr's compressibility index values were found to be less than 15% and Hausner's ratio values were also found to be lesser than 1.25, hence it indicates better flow properties. Thus, the prepared powder blend was found to exhibit good flow properties as evident from the results.

Evaluation of prepared mini-tablets of rifampicin and isoniazid

The weight variation values for all the prepared mini-tablets were found to range between 23±0.16 to 27±0.20 mg. As per United state pharmacopoeia (USP), the limit for percentage deviation of tablets of 130 mg or less is ±10%. Hence, in order to pass the weight variation test for all the prepared batches, ±2.5 mg (i.e. ±10%) is considered as accepted for 25 mg mini-tablets. Thus, all the RMT, IMT batches were found to pass as per the given specifications in USP. The Hardness values were found to be uniform and were range between 2.18±0.14 to 2.36 ± 0.08 kg. The values of Friability were found to range between 0.38±0.17 to 0.58 ± 0.08 % and they have also shown that mini-tablets have got sufficient strength. The Thickness values were found to range between 2.12±0.01 to 2.24±0.02 mm. Excellent drug content uniformity was found in the all the RMT and IMT mini-tablet batches, as their values were found to range between 99.17±0.11 to 99.90±0.24 % which is more than 95%. Thus, all the physico-chemical properties of mini-tablet batches were found to be satisfactory as evident from the results.

***In-vitro* buoyancy and lag time study**

In the present reported formula of floating RMT, various viscosity grades and concentrations of hydrophilic polymer (i.e. HPMC-K4M and HPMC-K100M) were used as matrixing agent, so as to sustain the drug release for a period of atleast 4 hours in pH 1.5 acidic media. Since, HPMC is one of the most commonly used hydrophilic polymer, which after wetting with water becomes swollen matrix. Whereas, sodium bicarbonate was incorporated in mini-tablets as a gas-generating agent. Moreover, citric acid was used as an anti-oxidant and buffering agent.

In this study, the main objective was to develop floating mini-tablets of rifampicin which shows less than 3 minutes as floating lag time and atleast 4 hour as floating time duration. So preliminary studies were carried out in 0.1 N HCl, by formulating four different placebo mini-tablet batches prepared with varying concentration of sodium bicarbonate (10%, 12%, 14%, 16%) along with constant concentration ratio of drug (40%) and polymer (30%). Finally from the *in-vitro* buoyancy and lag time study, it was found that RMT mini-tablets containing 16% sodium bicarbonate have shown desired lag time to float (i.e. 2.4 minutes) and floating time duration (i.e. 4.4 hours). This is due to the reason that effervescent mixture in RMT mini-tablets produced CO_2 which was trapped in swollen matrix, thereby decreasing the density of the mini-tablet below 1, thus making them buoyant.

***In-vitro* dissolution studies for rifampicin Liquid dispersion-filled capsule batches (RLD)**

The RLD batches were prepared by varying concentrations (0%, 5%, 10%, 15%) of PVP-K30 as viscosity modifier, so as to maintain the gel consistency and sustain the release of rifampicin. For all the RLD batches, liquid dispersion equivalent to 100 mg of rifampicin (i.e. 0.3 ml) were filled into the size "0" HPMC capsule. Further, all the RLD filled capsule batches were individually subjected to *In-vitro* dissolution testing in order to find out the effect of PVP-K30 in liquid dispersion. The objective was to select the most optimized RLD batch which can sustain rifampicin release for atleast 4 hours and can be successfully combined with the optimized IMT batch so as to prepare the novel

capsule-in-a-capsule technology. From the results of *in-vitro* dissolution studies as represented in Figure 4(a), it was found that RLD-1, RLD-2, RLD-3, RLD-4 batches released 98.46±0.98 %, 98.13±1.09 %, 99.34±1.15 % and 99.12±0.85 % of rifampicin at the end of 12, 12, 15 and 20 minutes respectively. The dissolution testing results have also shown that as the concentration of PVP-K30 was increased from 0% to 15% the release rate of rifampicin from liquid dispersion batches was decreasing. This is due to the high viscosity nature of liquid dispersion batches because of PVP-K30 which has delayed the drug release. However, it was found that all the RLD batches could not sustain rifampicin release as per our desired criteria. Moreover, during formulation development it was also observed that the concentration of PVP-K30 cannot be increased beyond 15% so as to prepare RLD liquid dispersions. It is due to the reason that when the concentration of PVP-K30 was increased, the liquid dispersion batches became more viscous and finally converted into paste. Hence, by observing the above failed results, our idea has transformed towards developing floating mini-tablets so as to sustain rifampicin release.

***In-vitro* dissolution studies for floating rifampicin mini-tablet batches (RMT)**

The RMT batches were prepared by varying concentration ratios of HPMC-K4M and HPMC-K100M (4:1, 3:2, 2:3 and 1:4) as release retardent polymers, so as to sustain the release of rifampicin in the 0.1 HCl (pH 1.2) for atleast 4 hours. Sodium bicarbonate and PVP K30 were incorporated in mini-tablets as a gas-generating agent and binder respectively. For all the RMT batches, mini-tablets equivalent to 100 mg of rifampicin (i.e. 10 number) were filled into the size "0" HPMC capsule. Further, all the RMT filled capsule batches were individually subjected to *In-vitro* dissolution testing in order to find out the effect of release retardent polymers in mini-tablets. The objective was to select the most optimized RMT batch which can be combined with the optimized IMT batch for preparing the novel capsule-in-a-capsule technology. From the results of *in-vitro* dissolution studies as represented in Figure 4(b), it was found that the RMT-1, RMT-2, RMT-3, RMT-4 batches released 99.94±0.68 %, 98.37±0.94 %, 99.92±72 % and 99.96±0.95 % of rifampicin at the end of 3, 4, 4 and 4 hours respectively. The results have also shown that as the concentrations and grade of the polymers in mini-tablets was increasing, the release rate of rifampicin was decreased. This is because the viscosity of the polymer has increased and hence retarded the drug release. Finally, after observing the release profile of all the RMT batches, RMT-4 was considered as the most optimized batch because its release profile was more sustained when comparing to all the remaining batches. Hence, HPMC-K4M and HPMC-K100M polymers when incorporated in mini-tablets in 30% concentration (1:4 ratio) along with 16% concentration of sodium bicarbonate has sustained the release of rifampicin in stomach for a period of 4 hours. Thus, RMT-4 floating mini-tablets batch was selected for developing a capsule-in-a-capsule formulation.

***In-vitro* dissolution testing for isoniazid mini-tablet batches (IMT)**

The IMT batches were prepared by varying concentrations of eudragit L100 (20%, 40%) and Eudragit S100 (20%, 50%) as pH dependent polymers, so as to prevent the release of isoniazid in the pH of stomach and to release only in small intestinal pH. For all the IMT batches, mini-tablets equivalent to 50 mg of Isoniazid (i.e. 5 numbers) were filled into the most suitable size "3" HPMC capsule. Further, all the IMT filled capsule batches were individually subjected to *in-vitro* dissolution testing in order to find out the effect of pH dependent polymers in mini-tablet batches. The objective was to select the most optimized IMT batch which can be combined with the optimized RMT batch so as to prepare the novel capsule-in-a-capsule formulation. From the results of *in-vitro* dissolution studies as represented in Figure 4(c), it was found that the IMT-1, IMT-2, IMT-3, IMT-4 batches released isoniazid after a lag time of 17, 21, 29, 123 minutes and 99.61±0.92 %, 99.97±0.74 %, 99.84±1.02 % and 99.93±0.96 % of isoniazid at the end of 4, 4, 5 and 6 hours respectively. The results have also shown that as the concentration of both the eudragit polymers in mini-tablets was increasing the release rate of isoniazid was decreasing. It was observed that IMT-1, IMT-2 batches prepared with 20%, 40 % concentrations of eudragit L100 polymer and IMT-3 batch prepared with 20% concentration of eudragit S100 polymer were not able to prevent the release of isoniazid in the pH of stomach. Whereas, the IMT-4 batch prepared with 50% concentration of eudragit S100 polymer was able to prevent the release of isoniazid in the pH of stomach. This is because the eudragit S100 polymer is having low solubility in pH 1.2, 6.5, 6.8 buffers and high solubility in pH 7.0 or above buffers. Hence, eudragit S100 polymer when incorporated in mini-tablets in high concentration (i.e. 50 %) has given a complete lag time in the pH of stomach, and thereafter sustained the release of isoniazid within the pH of small intestine. Finally, after observing the release profile of all the IMT batches, IMT-4 was considered as the most optimized because its release profile was superior to all the remaining batches. Thus, IMT-4 mini-tablets batch was selected for developing a capsule-in-a-capsule formulation.

***In-vitro* dissolution testing of capsule-in-a-capsule formulation of rifampicin and isoniazid**

Finally, after observing the seperarate *in-vitro* dissolution testing results of liquid dispersions and Floating mini-tablets of rifampicin and isoniazid mini-tablet batches, IMT-4 and RMT-4 were considered as optimized and were filled into a smaller (size "3") and a bigger HPMC capsule (size "0") respectively for developing capsule-in-a-capsule formulation. Further, the capsule-in-a-capsule formulation was also subjected to *in-vitro* dissolution

studies to assess the release of both the drugs (rifampicin and isoniazid) when administered in combination. From the results of *in-vitro* dissolution studies as represented in Figure 4(d), it was found that capsule-in-a-capsule formulation released 96.92±1.14 % of rifampicin at the end of 4 hours and 4.17±1.68 %, 99.06±1.88 % of isoniazid was released at the end of 2 and 6 hours respectively. The above developed capsule-in-a-capsule formulation have successfully released rifampicin and isoniazid in the pH of stomach and small intestine respectively as observed from the *in-vitro* results. Moreover, rifampicin and isoniazid were not released simultaneously in 0.1N HCL (pH 1.2), as it is observed that only 4.17±1.68 % of isoniazid was released at the end of 2 hours which can be considered as lag time. Thus, rifampicin and isoniazid can be used as combination product in the form of capsule-in-a-capsule formulation for the treatment of Tuberculosis.

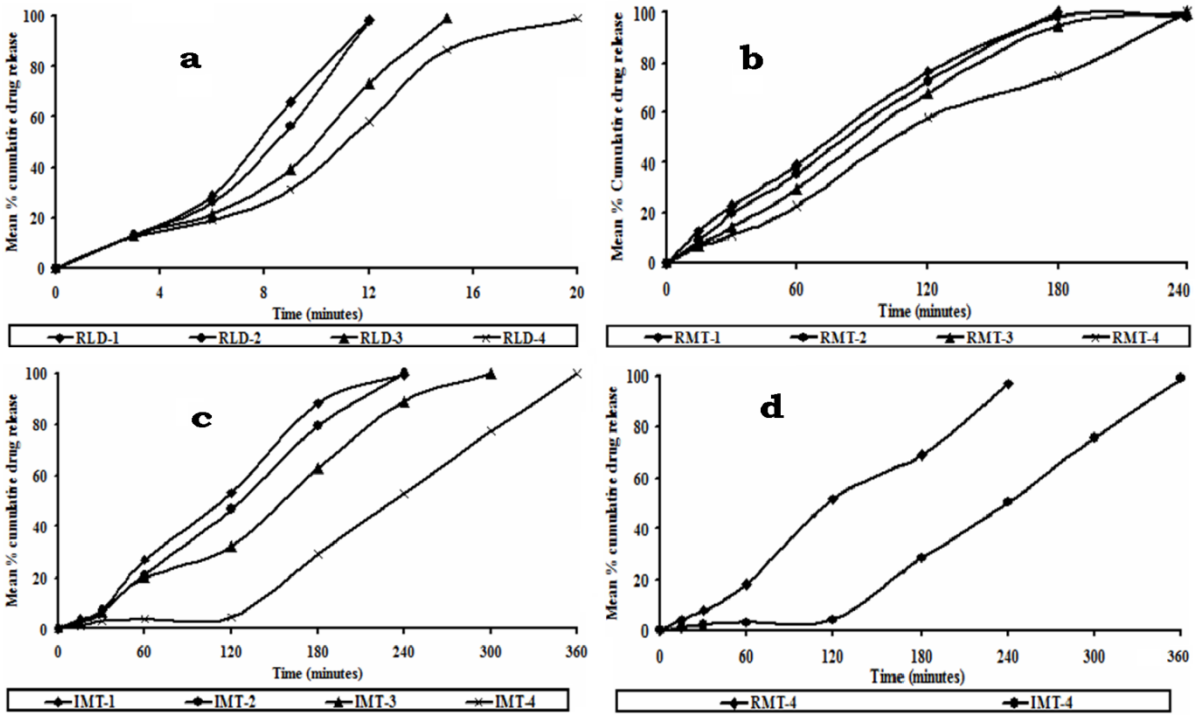

Figure 4: *In-vitro* release profile of a) rifampicin liquid dispersion (RLD) batches b) rifampicin mini-tablet (RMT) batches c) isoniazid mini-tablet (IMT) batches d) capsule-in-a-capsule formulation of rifampicin and isoniazid

Stability studies

The obtained results of stability studies at both room temperature and accelerated stability revealed that there was very small variation (i.e., < 1 %) in post-compression parameters, drug content and *in-vitro* drug release profile of the optimized capsule-in-a-capsule formulation. The observed results have not shown any significant changes in all the parameters of rifampicin matrix mini-tablets (RMT-4) and isoniazid matrix mini-tablets (IMT-4) batches during the 6 month period of study. Thus for the developed capsule-in-a-capsule formulation, stability was found as per ICH guidelines.

CONCLUSION

A novel capsule-in-a-capsule technology using multiple unit mini-tablets was successfully developed to sustain and target the release of rifampicin in stomach and isoniazid in small intestine. The desired results were confirmed using *in-vitro* dissolution studies. This formulation was also found to be stable as per ICH guidelines. From the industrial point of view, mini-tablets are superior to all other multiple unit dosage forms in terms of manufacturing, product quality and economical aspects. Whereas, capsule-in-a-capsule technology can be used for combination of dual release incompatible drugs for targeting to two different regions of gastro-intestinal tract. Thus, this work reflects the importance of a novel technology for delivering combination drugs with improved functionality. This technology can be of ultimate importance and also profitable investment for pharmaceutical industries especially in the developing countries.

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REFERENCES

1. Gohel MC, Sarvaiya KG, Nagori SA. Design and evaluation of novel dosage form of Rifampicin and Isoniazid with improved functionality. Indian Journal of Pharmaceutical Education and Research, 44(1), 2010, 22-27.
2. Ellard GA, Fourie PB. Rifampicin bioavailability: a review of its pharmacology and the chemotherapeutic necessity for ensuring optimal absorption. The International Journal of Tuberculosis and Lung Disease, 3, 1999, S301–S308.
3. Shishoo CJ, Shah SA, Rathod IS, Savale SS. Impaired Bioavailability of Rifampicin from its Fixed Dose Combination (FDC) Formulations with Isoniazid. Indian Journal of Pharmaceutical Sciences, 63, 2001, 443-449.
4. Singh S, Mariappan T, Sharda N, Kumar S, Chakrabarti A. The reason for an increase in decomposition of Rifampicin in the presence of Isoniazid under acid conditions. Pharmacy and Pharmacology Communications, 6, 2000, 405-410.
5. Singh S, Mohan B. A pilot stability study on anti-tuberculosis four drug fixed dose combination products. The International Journal of Tuberculosis and Lung Disease, 7, 2003, 298-303.
6. Bhutani H, Mariappan TT, Singh S. A study on the physical and chemical stability of anti-tuberculosis fixed-dose combination (FDC) products under accelerated climatic conditions. The International Journal of Tuberculosis and Lung Disease, 8, 2004, 1073-1080.
7. Singh S, Mariappan TT, Sankar R, Sarda, N, Singh B. A critical review of the probable reasons for the poor/variable bioavailability of rifampicin from anti-tubercular fixed-dose combination (FDC) products, and the likely solutions to the problem. International Journal of Pharmaceutics, 228, 2001, 5–17.
8. Laserson KF, Kenyon AS, Kenyon TA, Layloff T, Binkin NJ. Substandard tuberculosis drugs on the global market and their simple detection. The International Journal of Tuberculosis and Lung Disease, 2001, 448-454.
9. Mariappan T, Singh S. Regional gastrointestinal permeability of Rifampicin and Isoniazid (alone and their combination) in the rat. The International Journal of Tuberculosis and Lung Disease, 7, 2003, 797-803.
10. Duocap Capsules. Broad range of possibilities. Capsugel, Now a Lonza company. Available at http://www.capsugel.com/consumer-health-nutrition-products/duocap-capsules
11. Srinivasa Rao A, Mohd Nayeemuddin, Mohd Abdul Hadi. Formulation and evaluation of a novel capsule-in-a-capsule technology for biphasic delivery of lornoxicam in the treatment of migraine. International Journal of Pharmaceutical and Biomedical Research, *4*, 2013, 170-176.
12. Lopes CM, Lobo JM, Pinto JF, Costa P. Compressed mini-tablet as a biphasic drug delivery system. International Journal of Pharmaceutics, 323, 2006, 93-100.
13. Dina Gaber M, Noha Nafee, Osama Abdallah Y. Mini-tablets versus pellets as promising multiparticulate modified release delivery systems for highly soluble drugs. International Journal of Pharmaceutics, 488, 2015, 86-94.
14. Lennartz P, Mielck JB. Minitabletting: improving the compactibility of paracetamol powder mixtures. International Journal of Pharmaceutics, 173, 1998, 75-85.
15. Jyothi Sanaboina, Maheswari KM, Seetha Sunkara, Sravanthi Deekonda, Buchi Nalluri N. Preparation and Evaluation of Valsartan Liquid Filling Formulations for Soft Gels. Journal of Pharmaceutics, 2013, 1-8.
16. Deepthi Y, Gopalakrishna Murthy TE. Design and development and evaluation of candesartan cilexetil liquid filling formulations. International Journal of Pharmaceutical Investigation, 5, 2015, 81–86.
17. Roopa rani Balivada. Solubilized formulation and evaluation of liquid filled hard gelatin capsules of estrogen receptor modulator drug. International Journal of Research in Pharmacy and Chemistry, 1, 2011, 1046-1057.
18. Hadi MA, Raghavendra Rao NG, Srinivasa Rao A. Matrix-mini-tablets of lornoxicam for targeting early morning peak symptoms of rheumatoid arthritis. Iranian Journal of Basic Medical Science, 17, 2014, 357–369.
19. Antipas AS, Landis MS. Solid-state excipient compatibility testing. In: Baertschi SW. (Ed.), Pharmaceutical stress testing predicting drug degradation, Informa Healthcare, New York, 2005, PP 419-458.
20. Lachman L, Lieberman A. The theory and practice of Industrial Pharmacy. Special Indian edition. 2009, 293-373.
21. Mahipal Reddy Donthi, Narendar Reddy Dudhipala, Devendhar Reddy Komalla, Dinesh Suram and Nagaraju Banala. Preparation and Evaluation of Fixed Combination of Ketoprofen Enteric Coated and Famotidine Floating Mini Tablets by Single Unit Encapsulation System. Journal of Bioequivalence and Bioavailability, 7, 2015, 279-283.
22. Ravi Kumar, Patil MB, Sachin Patil R, Mahesh Paschapur S. Formulation and Evaluation of Effervescent Floating Tablet of Famotidine. International Journal of PharmTech Research. 1, 2009, 754-763.
23. Evans DF, Pye G, Bramley R, Clark AG, Dyson TJ, Hardcastle JD. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut, 9, 1988, 1035-1041.
24. Newton, Maria John A, Prabakaran L, Jayaveera KN. Formulation development, optimization and study on drug release kinetics of Eudragit® L100-HPMC E15 LV mixed film-coated colon-targeted Mesalamine tablets. Asian Journal of Pharmaceutics, 6, *2012,* 180-189.
25. Cha J, Gilmor T, Lane P, Ranweiler JS. Stability studies in Handbook of modern pharmaceutical analysis. Separation Science and Technology. Elsevier, 2001, 459-505.
26. ICH Guidelines, 2003. Stability testing of new drug substances and products. Q1A (R2) Step 4 versions. Available at: http://www.ich.org/cache/compo/363-272-html #Q1C. http://www.isppharmaceuticals.com Literature/ ISPPH5951_Advantia_Perf_Case_Study_VF.pdf.

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