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FORMULATION OF METFORMIN HYDROCHLORIDE SUSTAINED RELEASE MATRIX TABLET AND VALIDATION BY IN-VITRO EQUIVALENCE STUDIES

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Abstract: Metformin Hydrochloride (MH) is a biguanide, used to treat type 2 diabetes. The aim was to develop a high efficacious MH Sustained Release (SR) tablet formulation, bioequivalent to innovator product, utilizing polymers as drug release controlling substances. Tablets of each formulation were prepared sequentially, through wet granulation technique using MH, maize starch, magnesium stearate, and different combinations of Eudragit RSPO, (Poly (ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride) 1 : 2 : 0.1), Hydroxypropyl methylcellulose and, Xantural 75 (Xanthan gum). All formulations were evaluated for weight variation, friability, hardness, thickness, diameter, the content of uniformity, and in vitro drug release. Biowaiver studies and accelerated stability studies were performed. The physical and chemical parameters of all developed formulations complied with BP and USP standards. The similarity between dissolution profiles of developed formulations and the innovator product was studied and evaluated through difference factor ($f_1$) and similarity factor ($f_2$) in pH 6.8, 4.5, and 1.2 media. The values of $f_1$ and $f_2$ in pH 6.8, 4.5, and 1.2 media for final formulation were $f_1 = 7.05$, $7.24$, $8.76$, and $f_2 = 66.75$, $66.55$, $62.41$ respectively. Release kinetics was performed, and drug release was fitted with the quadratic model. The developed chemically and physically stable MH SR tablet formulation had equivalence of in vitro performance to innovator product according to international standards.

Keywords: Metformin Hydrochloride (MH), British Pharmacopoeia (BP), United States Pharmacopoeia (USP), biowaiver studies, difference factor ($f_1$), similarity factor ($f_2$)

Type 2 diabetes mellitus (T2DM) is a non-communicable disease (1) and causes disability and death worldwide. T2DM is described as non-insulin-dependent diabetes mellitus (2), where increased blood glucose level is due to comparatively less amount of insulin secretion or insulin resistance or decreased insulin action on target tissues (3).

MH is a biguanide (3), used to treat type 2 diabetes through oral administration as a first-line drug choice (4-6). MH is a hydrophilic drug, which facilitates glucose utilization by peripheral tissue, reduces glucose production in the liver through inhibition of gluconeogenesis and decrease glycogenolysis, and restricts sugar absorption in the small intestine (3, 6-7). It does not induce beta cells for secretion of insulin, and therefore does not cause hypoglycemia (3).

MH is absorbed into blood mainly in the small intestine; a high extent in the duodenum and jejunum, and a lesser extent in the ileum (8). Less time for drug absorption at the absorption site leads to reduced efficacy of the drug (9). The presence of food in the stomach will increase gastric emptying time. Increased residential time of drug in the stomach or small intestine leads to increased bioavailability (10).

Metformin has a bioavailability of 50-60% and a half-life of 1.5 to 4.5 hours (6, 11). The pKa of metformin is 12.4 and the pH is 6.68 (12). The peak plasma concentration ($C_{max}$) for an immediate release metformin tablet is achieved between one to three hours. In the case of a sustained-release dose, it takes four to eight hours. The apparent volume of distribution is very high for MH (300 to 1000 liters for a single dose) and plasma protein binding is negligible. MH is not metabolized in the body, and it is eliminated in unchanged form in urine by

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tubular secretion (13). Conventional dosage of MH needs repeat administration for efficient treatment (500 mg three times per day) (5, 11). Frequent administration and side effects of MH lead to decreased patient compliance (6). The dose of sustained-release formulation is the same as conventional formulation, but the frequency of administration can be minimized (14). Plasma levels of MH would be maintained for 8-12 hours by a single oral sustained-release tablet (6). It may suffice as a single daily dose (5, 6, 15).

The main drug-releasing mechanisms of controlled release products are diffusion, swelling, and erosion (16). Hydroxypropyl methylcellulose (HPMC), Xantural 75, and Eudragit RSPO are hydrophilic polymers. The hydrophilic polymers control drug release from dosage form through the mechanism of hydrogelation (17). The gel layer formation and resultant drug release rate are controlled by water penetration, polymer swelling, drug dissolution, diffusion, and matrix erosion. The formation and stability of the gel layer show the kinetics of drug delivery of the matrix system, which is limited by the chemical structure, concentration, and viscosity of polymers (18).

The Eudragit RSPO is a pH-independent, cationic synthetic polymer (19-22). It functions as a binder in the granulation process. During compression of granules, it develops a matrix structure to facilitate sustained release (23).

HPMC is a very low toxic and non-ionic hydrophilic polymer, widely used in oral sustained-release formulations due to high swelling ability, better compression, and gelling characteristics (6, 16, 18). When HPMC incorporated drug is dipped in water or biological fluid, the water diffuses into the polymer and relaxes the polymer chain, and forms a gel. The drug diffuses through the gel and reaches the medium (16). The release of the drug from the polymer is regulated through controlling swelling and cross-linking (6).

Natural polymer materials have been used in the development of sustained-release dosage forms over several years (12). Xantural 75 is a natural anionic polymer with 75 µm particle size (18, 24). It is a high molecular weight polysaccharide, having a cellulose backbone and trisaccharide side chains of mannose-glucuronic acid-mannose on alternating residues (17, 25). It is produced by the fermentation process of carbohydrates with gram-negative bacterium Xanthomonas amestris (26). The anionic nature of polymer is due to the presence of glucuronic acid and the pyruvic acid group on the terminal mannose in the side chain (25).

In vitro dissolution is an important tool in the development of solid drug dosage forms (27). The release of drugs from the system involves both dissolution and diffusion processes (28). There are several kinetic models to illustrate the drug release pattern from immediate and sustained-release dosage forms (27), such as Zero-order kinetics, First-order kinetics, Hixon-Crowell model, Higuchi model, Weibull model, Baker-Lonsdale model, Korsmeyer-Peppas model, Power law, and Hopfenberg Model.

This study describes the development of a sustained-release (SR) matrix tablet of high efficiency using hydrophilic polymers. The main objective was to produce a tablet bioequivalent to innovator product at an affordable price for the treatment of diabetic and prediabetic patients according to international standards. The assay, physical properties, and in-vitro drug release characteristics were evaluated according to British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP).

The in vitro equivalence (biowaiver) study was performed for the developed product with the innovator of MH sustained-release tablet at the end of the successful formulation development of MH SR tablet.

EXPERIMENTAL

Materials

The MH, Eudragit RSPO, HPMC, Xantural 75, maize starch, and magnesium stearate were used to develop the MH SR tablet. All these materials were obtained from Astron Limited, Ratmalana, Sri Lanka. MH, Magnesium stearate and maize starch complied with the BP (29) standard as well, Eudragit RSPO, HPMC, and Xantural 75 complied with the USP standard (30). Glucophage MH tablet (Merck Sante, France) was used as a control tablet (innovator product). Analytical grade chemicals (dibasic sodium phosphate, monobasic sodium phosphate, phosphoric acid, sodium acetate, acetic acid, sodium hydrochloride, and hydrochloric acid) were used for dissolution studies and spectroscopy analysis. High-Performance Liquid Chromatography (HPLC) grade chemicals (sodium 1-heptanesulfonate, sodium chloroacetic acid, and phosphoric acid) and MH standard USP were used for analysis by HPLC. The 130 µm thickness cold forming base foil and aluminum foil with 20 µm film thickness were used for Alu/Alu packing of the MH SR tablets. The high-density polyethylene (HDPE) containers were used for bulk packing.
Evaluation of the behavior of MH in different pH media

MH standard solutions were prepared in pH 6.8, 4.5, and 1.2 media, and the λ\text{max} for metformin was determined by wavelength scan in UV-Visible spectrophotometer. The concentration of metformin for the acceptable absorbance value at 232 nm was estimated in pH 6.8, 4.5, and 1.2 media.

For the biowaiver study, standard curves for metformin at 232 nm were plotted at three different pH values of 6.8, 4.5, and 1.2 to account for changes in the variation of absorbance of metformin with pH.

The standard solutions of MH were prepared in three different pH media and stored at 25°C ± 2 for 19 hours and variation in absorbance was studied over 19 hours. Aliquots were withdrawn from six dissolution vessels and the variation in absorbance was studied over 19 hours.

Preparation of tablets

The different formulations were prepared by the wet granulation technique according to Table 1. Maize starch was used as the binder, Eudragit RSPO, HPMC, and Xantural 75 were used as drug release controlling polymers, and magnesium stearate was used as a lubricant. The wet granules were dried and milled to get suitable particles in size for tablet compression. Dried granules were mixed with magnesium stearate and tablets were compressed in a tablet compression machine. The tablet compression process was performed at 23°C ± 2 temperature and 50% ± 5 relative humidity.

Evaluation of physical properties of trial tablet batches and innovator product

Physical parameters of developed formulations and innovator product were evaluated as follows.

Uniformity of weight

The weight of 20 tablets taken randomly was measured individually on an analytical balance. The individual weight must be within 5% of the average weight (29).

Friability of tablets

Ten tablets were taken and loose dust on tablets was removed. The weight of the tablet sample was measured accurately and placed in the pan of a tablet friability apparatus. The pan was rotated 100 times and any loose dust from the tablets was removed. The weight of the tablet sample was measured accurately.

\[
\text{Percentage of weight loss} = \frac{(\text{Initial weight of tablets} - \text{Final weight of tablets})}{\text{Initial weight of tablets}} \times 100
\] (1)

The percentage of weight loss must be less than 1% (30).

Hardness test

Twenty tablets were taken randomly from each batch. The hardness of individual tablets was measured by placing the tablet between the fixed jaw and the sliding jaw of the tablet hardness tester (6, 31).

Thickness measurement

Twenty tablets were taken randomly from each batch. The thickness of individual tablets was measured by placing the tablet between the working platform and the indenter of the tablet hardness tester (6, 31).

Diameter Measurement

Twenty tablets were taken randomly from each batch. The diameter of individual tablets was measured by placing the tablet between the fixed jaw and the sliding jaw of the tablet hardness tester.

| Raw materials      | Formulation (weight per tablet in mg) |
|-------------------|---------------------------------------|
|                   | A  | B   | C   | D   | E   | F   | G   | H   |
| MH                | 510.1 | 510.1 | 510.1 | 510.1 | 510.1 | 510.1 | 510.1 | 510.1 |
| Eudragit RSPO     | 60.0   | 100.0   | 40.0   | 102.0   | 0.0   | 0.0   | 0.0   | 0.0   |
| HPMC              | 100.0  | 60.0  | 60.0  | 170.0  | 230.0  | 271.0  | 257.5  | 250.0  |
| Xantural 75       | 40.0   | 40.0   | 100.0  | 68.0   | 100.0  | 94.0   | 100.0  | 110.0  |
| Maize starch      | 55.0   | 55.0   | 55.0   | 61.1   | 60.4   | 53.8   | 62.3   | 62.6   |
| Magnesium stearate| 5.0    | 5.0    | 5.0    | 6.0    | 6.0    | 6.0    | 6.0    | 6.0    |

MH assay of developed formulations and innovator product

The MH assay was performed through HPLC analysis. The buffer solution was prepared with 0.5 g/L of sodium 1-heptanesulfonate and 0.5 g/L of NaCl and pH was adjusted to 3.85 with 0.06 M phosphoric acid. The acetonitrile and buffer were mixed in 1 : 9 ratio for the mobile phase. A 1.25% acetonitrile solution was used as a diluent. The 0.125 mg/mL MH reference standard was prepared in the diluent.

The 20 tablets of MH SR were finely powdered and an equivalent to the average tablet weight of powder was transferred to the homogenization vessel and 500 mL of 10% acetonitrile solution was added. The mixture was homogenized and allowed to soak until the sample was fully homogenized. The 25 mL of filtered sample solution was transferred to 200 mL of volumetric flask and diluted with water up to volume.

The L1 (3.9 mm x 30 cm x 10 µm packing) liquid chromatography column was used for analysis at 30ºC and UV 218 nm detection. The 10 µL of volume was injected at the flow rate of 1 mL/min. The amount of MH per tablet was calculated against the standard (30).

In vitro drug release studies

In vitro drug release studies were carried out according to dissolution test 8 of USP with dissolution tester. The 1000 mL of pH 6.8 phosphate buffer was used for the dissolution test with apparatus 2 at 100 rpm. At the end of 1, 2, 4, 6, 8, and 10 hours, the sample was withdrawn, and cumulative drug release was calculated through the measurement of absorbance at 232 nm in a UV-Visible spectrophotometer. The MH standard in the medium was used as standard. According to USP, cumulative drug release percentage at the end of 1, 2, 6, and 10 must be 20-40%, 35-55%, 65-85%, and not less than 85% respectively (30).

Drug release kinetics

Drug release kinetics was matched with various models to explain the mechanism. In vitro drug release data were fitted into zero-order, Higuchi, and Quadratic models. The drug release kinetics was analyzed in SAS statistical software.

Zero-order model: \( Q_i = Q_0 + K_0 t \)  \( \text{(2)} \)

Where \( Q_i \) is the amount of drug dissolved in time \( t \), \( Q_0 \) is the amount of drug in the solution at the initial stage and \( K_0 \) is the zero-order release constant (27, 32).

Higuchi model: \( Q = K_H T_1^2 \)  \( \text{(3)} \)

Where, \( Q \) is the amount of drug release at time \( T \) and \( K_H \) is the Higuchi rate constant (6, 27).

Quadratic model: \( Q = m_1 t^2 + m_2 t + c \)  \( \text{(4)} \)

Where \( Q \) is the amount of drug release at time \( t \), \( m_1 \) and \( m_2 \) are model parameters and \( c \) is intercept (33).

Movement of dissolution profiles

The model-independent approaches drive a single value from the dissolution profile which was used to compare the dissolution data directly. The common ratio test is a comparison of two mean dissolution times (MDTs).

\[ \text{MDT} = \frac{\sum_i (t_i - MDT)^2 \Delta M_i}{\sum_i \Delta M_i} \]  \( \text{(5)} \)

Where \( i \) is the sample number, \( n \) is the number of dissolution sample times, \( t_i \) is the time at a midpoint between \( t_{i-1} \) and \( t_i \), and \( \Delta M_i \) is an additional amount of drug dissolved between \( t_{i-1} \) and \( t_i \).

The variance of dissolution times (VTs) was calculated by,

\[ \text{VT} = \frac{\sum_i (t_i - MDT)^2 \Delta M_i}{\sum_i \Delta M_i} \]  \( \text{(6)} \)

The relative dispersion of dissolution times (RD) was estimated by,

\[ \text{RD} = \frac{\text{VT}}{\text{MDT}^2} \]  \( \text{(7)} \)

Dissolution equivalency

The similarities and dissimilarities between the drug release profiles of developed SR formulation (test product) and the innovator product (reference product) were found according to FDA through similarity factor \( (f_2) \) (27, 36).

\[ \text{Similarity Factor: } f_2 = 50 \log \left\{ 1 + \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right\} \times 100 \]  \( \text{(8)} \)

Where \( n \) is the number of pull points, \( R_i \) is the reference profile at time point \( t \), and \( T_i \) is the test profile at the same time point, the value of \( f_2 \) should be between 50 and 100. If the \( f_2 \) value is 100, it indicates that the test and reference release profiles are identical. As the value of \( f_2 \) becomes smaller, the dissimilarity between test and reference release profiles increases (37).
The Difference factor \( (f_1) \) describes the relative error between the dissolution profile of the test and the reference product.

\[
\text{Difference Factor: } f_1 = \left( \frac{\sum_{i}^{n} \left| T_{i} - R_{i} \right|}{\sum_{i}^{n} R_{i}} \right) \times 100\% \tag{9}
\]

The percent error between the two curves was approximated by the difference factor \( (f_1) \). When the percent error is zero, the test and reference profiles are identical, and it increases proportionally with the dissimilarity between the two profiles (34).

**In-vitro equivalence studies (Biowaiver Studies)**

The dissolution test of 12 dose units was done for reference (innovator) and test product in three media as pH 1.2, pH 4.5, and pH 6.8 at 37ºC. The sampling time was 1, 2, 4, 6, 8, and 10 hours. And similarity factor \( (f_2) \) was calculated. The similarity factor \( (f_2) \) value of 50 or greater (50-100) shows the equivalence of two curves and the equivalence of *in vitro* performance of reference and test product (38).

**Stability studies**

According to the International Council for Harmonization (ICH) guidelines, tablets of optimized formulation (formulation H) were considered for stability studies. The tablets from specific formulation were packed in Alu-Alu blister pack and bulk pack in a plastic container with polythene bag under controlled environmental conditions of 24°C ±1 and 50% ±5 of RH. They were stored in a stability chamber at 40°C ±2 and 75% ±5 of RH condition for 6 months and then the physical and chemical properties of tablets were studied in 3 and 6 months (14, 39).

**RESULTS AND DISCUSSION**

**Variation of absorption maxima and stability of MH in different pH media**

The absorption maxima of MH varied with the pH of the medium. A single absorption maximum at 205 nm was obtained at pH 1.2. At pH 4.5 the single absorption peak appeared at 232 nm. However, at pH 6.8, two absorption maxima at 198 and 232 nm were observed with the peak at 198 nm predominating.

Metformin is a basic molecule. The primary and secondary amine groups (-NH2 and –NH group), are protonated to –NH3 + and –NH + respectively according to the concentration of H + in the medium. Due to this reason, \( \lambda_{\text{max}} \) varied in different pH media. A higher concentration of Metformin was used in pH 1.2 medium to get acceptable readings for absorbance at 232 nm.

The value for \( R^2 \) was 0.999 for standard curves in the three different pH media, showing that the absorbance for MH had a linear correlation with concentration. The concentration of MH in pH 1.2 medium was higher (≈ 3.5 times) than in pH 4.5 and 6.8 media for the same absorbance value, because of the increased protonation of the Metformin molecule at low pH.

**Table 2. Variation in absorbance of MH standard and sample solution at initial and after 19 hours in pH 6.8, 4.5, and 1.2 media with time.**

| MH          | In pH 6.8 medium | In pH 4.5 medium | In pH 1.2 medium |
|-------------|------------------|------------------|------------------|
|             | Initial          | After 19 hrs.    | Initial          | After 19 hrs.    | Initial          | After 19 hrs.    |
| Standard    | 0.539 ± 0.0019   | 0.538 ± 0.0013   | 0.521 ± 0.0027   | 0.521 ± 0.0026   | 0.509 ± 0.0034   | 0.508 ± 0.0034   |
| Sample solution | 0.476 ± 0.0055  | 0.475 ± 0.0060  | 0.468 ± 0.0026   | 0.475 ± 0.0032   | 0.518 ± 0.0062   | 0.520 ± 0.0058   |

*Mean value of six absorbance readings with ± standard deviation

**Table 3. Physical properties and MH assay of developed formulations and innovator product.**

| Formulation | Weight (mg) | Friability (%) | Thickness (mm) | Diameter (mm) | Hardness (kp) | Assay (mg/Tablet) |
|-------------|-------------|----------------|----------------|---------------|---------------|-------------------|
| A           | 775.9 ± 11.95 | 0.235         | 6.87 ± 0.08   | 16.97 ± 0.01  | 26.92 ± 0.67  | 506.83 ± 2.06    |
| B           | 780.5 ± 8.81  | 0.248         | 6.85 ± 0.07   | 16.89 ± 0.02  | 25.70 ± 0.73  | 504.78 ± 1.62    |
| C           | 761.5 ± 6.05  | 0.152         | 6.60 ± 0.04   | 16.95 ± 0.01  | 26.97 ± 0.50  | 504.10 ± 0.29    |
| D           | 906.7 ± 9.9   | 0.279         | 7.99 ± 0.06   | 17.00 ± 0.02  | 27.88 ± 1.56  | 497.46 ± 3.38    |
| E           | 901.7 ± 8.1   | 0.281         | 6.77 ± 0.05   | 17.00 ± 0.03  | 17.25 ± 0.90  | 491.05 ± 3.14    |
| F           | 958.1 ± 9.7   | 0.230         | 7.31 ± 0.06   | 17.03 ± 0.03  | 14.01 ± 0.51  | 502.46 ± 1.81    |
| G           | 963.3 ± 12.0  | 0.326         | 7.37 ± 0.07   | 16.96 ± 0.05  | 20.03 ± 1.19  | 502.19 ± 4.11    |
| H           | 950.5 ± 6.5   | 0.254         | 7.10 ± 0.04   | 17.00 ± 0.02  | 20.18 ± 0.80  | 495.16 ± 1.67    |
| Innovator product | 1036.9 ± 11.0 | 0.006         | 6.76 ± 0.02   | 19.13 ± 0.05  | 24.56 ± 0.68  | 494.72 ± 3.10    |

*Mean value with ± standard deviation
The stability of MH over 19 hours was determined in the three different pH media. The absorbance at 232 nm was measured in standard solution and six sample solutions respectively. There was no significant variation in the absorbance of MH in standard solution or the absorbance of MH in sample solutions in different pH media over 19 hours (Table 2). It shows Metformin was stable in standard and sample solutions of pH 6.8, 4.5, and 1.2 for 19 hours. In addition, Metformin was compatible with excipients in sample solutions of pH 6.8, 4.5, and 1.2 for 19 hours.

**Physical testing for tablets of developed formulations and innovator product**

Physical parameters such as weight, friability, thickness, diameter, and hardness for the tablets of developed formulations and innovator product were evaluated. The formulations A to H were developed sequentially, according to the drug-releasing pattern of the previous formulation. Each formulation had the same amount of MH but the amount and composition of polymers and the total weight of the tablets varied.

According to the BP (29), the weight variation for tablets greater than 250 mg must be within 5% of the average weight. The tablets of all developed formulations and the innovator product complied with BP limits having variations within 3% of average weight (Table 3). The friability test for all developed formulations and the innovator product was below 0.4% in compliance with USP (30), value of < 1%. The thickness and diameter have an impact on blister packing. These two parameters should be within acceptable limits for successful blister packaging. All sample formulations had comparable thickness and diameter to the innovator product. The strength of tablets was measured through a hardness test. Tablets that have low hardness, have a high possibility to break or damage in processing and handling. At the same time, tablets that have high hardness, have low disintegration. The hardness of tablets of all developed formulations and the innovator product were within acceptable limits. All these physical properties were obtained through the combination of binder and lubricant and the processing of granulation. The optimum setting of the tablet compression machine gave the proper hardness and friability.

**Assay for MH in developed formulations and innovator product**

The amount of Active Pharmaceutical Ingredient (API) in individual tablets (blend uniformity) is given by USP (30) as > 450 mg and < 550 mg of MH per tablet. The blend uniformity of developed formulations and the innovator product was evaluated through the MH assay of tablets.

All formulations were tested according to the USP standard. Tablet assay of all formulations lay between ± 2.5% of label claim. The maize starch paste was added after proper dry mixing of the blend for granulation. This process maintained the homogenization of the API in the blend.

**Drug Release patterns of developed formulations and the innovator product in different pH media**

The drug release patterns of compressed tablets (Formulations A-G) were studied through dissolution tests in pH 6.8 medium according to USP standard (30). Results are shown and illustrated in Figure 1. The drug-releasing pattern of formulations A, B, C, and D did not comply with USP standard (30) at pH 6.8. These formulations had higher cumulative drug-releasing properties than the standard due to the composition of polymers. To control the drug-releasing property, Eudragit RSPO was removed and the amount of HPMC and Xantural 75 was increased in the formulations of E, F, G, and H.

However, formulations E, F, and G complied with the drug-release pattern of the USP standard. The prolonged drug releasing property increased through formulation A to G with the change of amount and combination of polymers. Formulations E, F, G, and H had different compositions of HPMC and Xantural 75. The xanthan gum expresses the

![Figure 1. Cumulative drug release % of formulation A, B, C, D, E, F, and G in pH 6.8 medium (n = 6).](image-url)
drug-releasing properties independent of pH and buffer species (17). The composition of polymers in formulation H gave optimum drug-releasing, physical and chemical properties for the formulation. The proper ratio of various levels of polymers in the formulation has a major impact on drug releasing (6).

For biowaiver studies, drug release patterns in pH 6.8, 4.5, and 1.2 media were tested for formulations E, H, and the innovator product. Patterns are shown in Figure 2 (formulation E) and Figure 3 (formulation H and innovator) respectively.

There was no significant variation in the drug-releasing pattern of formulation E in pH 6.8, 4.5, and 1.2 media (Fig. 2). The HPMC is a non-ionic hydrophilic polymer and Xantural 75 is an anionic polymer (18, 24). The combination of these polymers gave the pH-independent drug-releasing property for the formulation.

The formulation H and innovator product had similar drug-releasing patterns in the different pH media with formulation H showing better drug-releasing property than innovator product.

**Drug release kinetics**

The drug release pattern of developed formulations and the innovator product in pH 6.8 medium were matched with Zero-order model, Higuchi model, and Quadratic model, and the value of $R^2$, adjusted $R^2$, Akaike’s Information Criteria (AIC), and Bayesian Information Criteria (BIC) were calculated through SAS statistical software. The results are shown in Table 4.

The value of adjusted $R^2$ close to 1.0 indicates that the specific release pattern matches that of the mathematical model. “The model with smallest AIC is deemed the best model since it minimizes the difference from the given model to the true model” (40). As shown in Table 4, the adjusted $R^2$ value of all developed formulations and the innovator product was close to 1.0 in the Quadratic model. At the same time, AIC values were also smaller in the Quadratic model. So, the drug-releasing patterns best fit with the Quadratic model.

**Movements of the dissolution profiles**

The Mean Dissolution Time (MDT) and Variance of Dissolution Time (VT) are ratio test parameters. MDT and VT can be used to compare different dissolution profiles and establish the correlation between in vivo and in vitro data (35).

The values of ratio parameters for preliminary formulations A-G in pH 6.8 medium showed an increase in MDT values with formulations G and H reaching 2.99 ±0.03 h and 3.02 ±0.05 h respectively. The innovator product had the highest MDT of 3.23 ±0.07 h. The MDT value indicates the drug-releasing ability of the formulation. A high MDT value means that the formulation has prolonged drug release properties (41). The final formulation H had high prolonged drug release properties compared to preliminary formulations A-G. The Formulation H released 94.2-96.5% of drug at the end of ten hours in the three different pH media while the innovator product released 89.9–92.0% of drug (Fig. 3). Therefore, formulation H is a better product than the innovator product in terms of efficient drug releasing property in ten hours.

**Fit factors and in vitro equivalence studies (Biowaiver studies)**

The difference factor ($f_1$) and similarity factor ($f_2$) are used to compare the dissolution profile of tablets (35, 41). The Food and Drug Administration (FDA) Center for Drug Evaluation and Research (CDER) suggested fit factors while similarity factor was suggested by the European Medicines Evaluation Agency (EMEA) Committee for the purpose of evaluation criterion of similarity between two in vitro dissolution profiles (41).

The $f_1$ evaluates the relative error between two dissolution profiles. When test and reference (innovator product) dissolution profiles are identical, the percentage error is zero. When dissimilarity between two dissolution profiles increases, the value of $f_1$ increases proportionally (34). The value of $f_2$ between 50 and 100 suggests test and reference (innovator product) dissolution profiles are similar.
Table 4. Values of $R^2$, adjusted $R^2$, AIC, and BIC for developed formulations and Innovator product in pH 6.8 medium.

| Formulation | Model   | $R^2$  | Adjusted $R^2$ | AIC    | BIC    |
|-------------|---------|--------|----------------|--------|--------|
| A           | Zero order | 0.9015  | 0.8768         | 28.0104| 31.5104|
|             | Higuchi   | 0.9687  | 0.9608         | 21.1334| 24.6334|
|             | Quadratic | 0.9975  | 0.9959         | 7.8533 | 13.8533|
| B           | Zero order | 0.8828  | 0.8535         | 27.8727| 31.3727|
|             | Higuchi   | 0.9578  | 0.9473         | 21.7446| 25.2446|
|             | Quadratic | 0.9945  | 0.9909         | 11.4890| 17.4890|
| C           | Zero order | 0.8918  | 0.8648         | 28.2362| 31.7362|
|             | Higuchi   | 0.9632  | 0.9540         | 21.7698| 25.2698|
|             | Quadratic | 0.9958  | 0.9931         | 10.6785| 16.6785|
| D           | Zero order | 0.9509  | 0.9386         | 23.3817| 26.8817|
|             | Higuchi   | 0.9930  | 0.9913         | 11.6507| 15.1507|
|             | Quadratic | 0.9973  | 0.9955         | 8.0098 | 14.0098|
| E           | Zero order | 0.9454  | 0.9318         | 23.8174| 27.3174|
|             | Higuchi   | 0.9910  | 0.9887         | 13.0154| 16.5154|
|             | Quadratic | 0.9984  | 0.9973         | 4.6882 | 10.6882|
| F           | Zero order | 0.9389  | 0.9237         | 24.5856| 28.0856|
|             | Higuchi   | 0.9884  | 0.9855         | 14.6115| 18.1115|
|             | Quadratic | 0.9974  | 0.9957         | 7.5761 | 13.5761|
| G           | Zero order | 0.9491  | 0.9364         | 23.2558| 26.7558|
|             | Higuchi   | 0.9926  | 0.9908         | 11.6663| 15.1663|
|             | Quadratic | 0.9983  | 0.9971         | 5.0234 | 11.0234|
| H           | Zero order | 0.9499  | 0.9374         | 23.5732| 27.0732|
|             | Higuchi   | 0.9927  | 0.9909         | 11.9956| 15.4956|
|             | Quadratic | 0.9986  | 0.9976         | 4.3055 | 10.3055|
| Innovator   | Zero order | 0.9568  | 0.9460         | 22.7566| 26.2566|
|             | Higuchi   | 0.9955  | 0.9944         | 9.1867 | 12.6867|
|             | Quadratic | 0.9979  | 0.9965         | 6.6389 | 12.6389|

Figure 3. Cumulative drug release% of formulation H and Innovator product in pH 6.8, 4.5, and 1.2 media (n=12).
product) dissolution profiles are similar. The $f_2$ value of 100 suggests that those dissolution profiles are identical. When dissimilarity between the two profiles increases, the value of $f_2$ becomes smaller (34). The $f_1$ value up to 15 and an $f_2$ value greater than 50, indicates that the average difference is less than 10% at each sample time point, which establishes the equivalence of two curves and consequences of the performance of the developed product and innovator product (41). The values of ratio parameters for preliminary formulations A-G in pH 6.8 medium are given in Table 5. The in vitro dissolution profiles of formulations D, E, F, G, and H were similar to the innovator product ($f_1 < 15$ and $f_2 > 50$).

WHO (38) states that for drug registration, a dossier (application) is acceptable based on bio-waiver studies other than in vivo equivalence testing. For the biowaiver studies, the dissolution profile for the developed product (formulation H) and innovator product must be carried out in pH 6.8, 4.5, and 1.2 media with a minimum of 12 dose units of each product (38). The $f_1$ value of formulation H was greater than 50 in pH 6.8, 4.5, and 1.2 media (Table 6). According to WHO specification, the in vitro performance of formulation H showed equivalence to the innovator product.

### Stability studies

The accelerated stability study was carried out for the shelf-life determination of the MH SR tablet. Formulation H was subjected to physical testing in the Alu-Alu pack and bulk pack over 3- and 6-month duration. The weight variation of tablets in accelerated stability complied with the BP standards. The friability of tablets in accelerated stability complied with the USP standards. The thickness, diameter, and hardness were within acceptable range throughout the accelerated stability studies. The MH assay of the tablet for the Alu-Alu pack and bulk pack complied with the USP standard containing 450-550 mg of API/tablet in stability studies at 3 and 6 months (Table 7).

The accelerated stability was performed for formulation H for 3 months and 6 months according to International Council for Harmonization (ICH) guideline (14, 39). The stability of formulation H complied with the standard. According to

| Formulation | Difference factor ($f_1$) | Similarity factor ($f_2$) |
|-------------|---------------------------|--------------------------|
| A           | 24.73                     | 39.90                    |
| B           | 25.92                     | 38.74                    |
| C           | 27.05                     | 37.98                    |
| D           | 13.23                     | 53.65                    |
| E           | 7.77                      | 64.53                    |
| F           | 7.59                      | 64.84                    |
| G           | 6.22                      | 68.93                    |

| Medium  | Difference factor ($f_1$) | Similarity factor ($f_2$) |
|---------|---------------------------|--------------------------|
| pH 6.8  | 7.05                      | 66.75                    |
| pH 4.5  | 7.24                      | 66.55                    |
| pH 1.2  | 8.76                      | 62.41                    |

| Type of packaging | Duration | Weight (mg) | Friabilty (%) | Thickness (mm) | Diameter (mm) | Hardness (kp) | Assay (mg/Tablet) |
|-------------------|----------|-------------|---------------|----------------|---------------|---------------|-------------------|
| Alu-Alu pack      | 3 months | 953.1 ± 4.6 | 0.08          | 7.19 ± 0.03    | 17.04 ± 0.01  | 25.83 ± 0.94  | 501.80 ± 4.86     |
|                   | 6 months | 952.3 ± 6.9 | 0.09          | 7.18 ± 0.04    | 17.06 ± 0.03  | 27.95 ± 1.32  | 497.39 ± 3.06     |
| Bulk pack         | 3 months | 955 ± 6.1   | 0.11          | 7.18 ± 0.03    | 17.07 ± 0.02  | 26.74 ± 0.84  | 495.16 ± 1.67     |
|                   | 6 months | 959.4 ± 5.7 | 0.12          | 7.20 ± 0.04    | 17.10 ± 0.02  | 27.53 ± 1.13  | 492.57 ± 2.25     |

*Mean value with ± standard deviation
USP standard (30), drug release from the tablet in 1st, 2nd, 6th and 10th hours must be between 20-40%, 30-50%, 65-85%, and not less than 85% respectively in pH 6.8 medium. The drug release pattern of formulations H in pH 6.8 medium complied with the standard over 6 months accelerated stability.

CONCLUSIONS

The physical testing and assay of all developed formulations complied with BP and USP standards. Drug releasing profile of formulation E, F, G, and H complied with USP standards. In consideration of difference factor ($f_1$) and similarity factor ($f_2$), Formulation G and H showed close similarity to the innovator product. The formulation H released a higher percentage of the drug than formulation G.

The $f_1$ value of formulation H was less than 9 and the $f_2$ value was greater than 62 in pH 6.8, 4.5, and 1.2 media. According to WHO guidelines, formulation H was similar to the innovator product. However, in terms of drug release, formulation H was more efficient than the innovator since at the end of ten hours a higher percentage of the drug was released from formulation H than from the innovator product. The results of biowaiver studies of formulation H complied with WHO standards. The stability study results show that this product was stable for 6 months in accelerated condition according to ICH guidelines and USP standards.

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Conflicts of interest

The author declares that he has no conflict of interest.

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