DEVELOPMENT AND EVALUATION OF FAST DISINTEGRATING EXTENDED RELEASE TABLETS CONTAINING ANTIHYPERTENSIVE DRUG

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ABSTRACT

Objective: The aim of this work was preparing once daily fast disintegrating tablets to handle easily for adult hypertensive patients who have difficulty in swallowing.

Methods: Solid dispersions bisoprolol hemifumarate (SD-BH) was prepared by using EC and HPMC in different ratios. A 3* 2 full factorial design was used to investigate the main formulation parameters (different fillers, binder differ in the molecular weight and different coat type).

SD-BH were prepared and characterized by DSC. Disintegration time, wetting properties, friability, and hardness of FDTs were evaluated. Percent drug dissolved was determined. Furthermore, the bioavailability was compared with commercial market product.

Results: The mean production yield of BH-SDs was 93.50 ± 0.39 %. The tablets demonstrated a hardness of 2-5 N, friability 0.04-0.56% and disintegration time of 67 ± 1.54 sec. The formulations were subjected to accelerated stability study as per ICH guidelines and were found to be stable after three weeks at 60 °C and 75 % R.H.

Conclusion: Based on The present study; the suggested FDTs (Ta1) which delivers a solid dispersions’ 10 mg BH using HPMC and EC in 1:1 ratio showed an extended effect in lowering the blood pressure and decrease the disintegrating time lesser than commercial oral tablets.

Keywords: Bisoprolol hemifumarate, Fast disintegrating tablets, Bioavailability study, Extended release, Solid dispersion.

INTRODUCTION

Hypertension is still one of the most significant risk factors for cardiovascular disease, especially in the adult population [1]. Thus; the development of an appropriate dosage form is desirable. Various changes in the physiological functions associated with aging as difficulty in swallowing large tablets or capsules or dysphagia made current dosage forms impractical [2].

The most desirable formulation for use by the elderly is one that’s easy to swallow and easy to handle [3]. Ease of swallowing and no need for water; only the small volume of saliva of these formulations, result in making fast disintegrating tablets’ primary benefit is improvement the patient compliance [4].

Drugs (β blockers) have been one of the primary treatment of hypertension because their ability to manage the heart failure [5]. Bisoprolol hemifumarate (BH) [(RS)-1-{1-[2-isopropoxyethoxy] methylphenoxy}-3-(isopropylamino)propan-2-ol] is a selective beta-1 receptor blocker. BH leads to 46% decrease in sudden death after one year of administration. BH administered is an effective and safe as antigniral agent. It acts essentially through reduction of myocardial oxygen consumption [6-7].

The administration of a drug in a controlled approach is being given greater attention for improved therapeutic levels. Sustained or controlled drug delivery occurs when the drug is released from the tablets at a constant rate for the required period. The polymer the drug or other active ingredients combined in such a way that help in delayed the release [8].

Solid dispersion (SD) technique has been applied for the controlled release of drugs. Previous reports have shown that by using SDs containing a polymer blend, such as hydroxypropyl methylcellulose (HPMC) and ethyl cellulose (EC), it is possible to precisely control the rate of release of an extremely water-soluble drug [9].

Hence, this study concerned with the development and evaluation a fast disintegrating, extended release tablets of BH as a new dosage form easy for handling of elderly hypertensive patients using a solid dispersion technique. Solid dispersion was prepared by the accession of a release-retarding polymer hydroxypropyl methyl cellulose (HPMC) and ethyl cellulose (EC) in different proportions. The effects of polymer loading on drug release recorded after studying the effect of formulae except on the disintegration time.

MATERIALS AND METHODS

Materials

BH was purchased from Merck (Barcelona, Spain). EC, HPMC, Ac-Oi-Sol (crosccarmellose sodium), magnesium stearate and aspartame were purchased from Fluka; Germany. Mannitol was purchased from El –Nasr Pharmaceuticals, Egypt. Anhydrous lactose, polyvinyl pyroldione, (PVP K30 and PVP K90) were purchased from Sigma; USA.

Methods

Preparation of solid dispersions batches (batches A, B, and C) by the solvent evaporation method

Solid dispersions BH were prepared by using EC and HPMC. Batches A, B and C were prepared using HPMC: EC ratio as 1:1, 1:2, and 2:1 respectively.

One gram of the drug was dissolved in a mixture of methanol and dichloromethane (1:1). Shaking very well was to ensure complete dissolving of the drug in the solvent mixture. Mixture of EC and HPMC equivalent to expected ratio pour in the solvent containing the drug. Stirring very well using mechanical stirrer and magnetic stirrer was till complete evaporation of the solvent. Sieving till give homogeneous powder by passed through a 200 μm sieve and retained on a 100 μm sieve. Subsequently, the sieved ground powders were stored at 25°C in a desiccator or in a screw-capped glass vial until use.

HPLC method for the determination of BH

The assay of BH was performed by a modified Joshi et al HPLC method [10]. A thermo Inertsil ODS 3V®, C18 column (5 μ, 25 cm × 4.6 mm, Hypersil) and a mobile phase consisting of 0.01 M
phosphate buffer (pH 7.4): acetonitrile (30:70 v/v) mixture was used at the flow rate was 1 ml/min, and the effluent was monitored at 273 nm.

The linearity, the precision, the selectivity, and the accuracy of the method with respect to intra- and inter-day for three days were demonstrated as per ICH guidelines [11].

Evaluation and characterization of SD batches

Percent yield of SDs and drug loading

The amount of the prepared batches A, B and C equivalent to 10 mg of BH were weighed accurately and washed with 100 ml of phosphate buffer PH 6.8 for 5 minutes. The extract was vortexed for 1 minute, followed by centrifugation at 10,500 g (Centrifuge 5810 R; Ependorf, Hamburg, Germany) for 10 minutes. The supernatant was filtered through a 0.45-μm membrane and the amount of BH determined by HPLC. Each sample was analyzed in triplicate. The formulation’s yield percentage was calculated using a calibration curve constructed of standard drug. The area of absorption in HPLC method was equivalent to the free drug content. The production yields of SDs (Y %) and drug loading (DL %) was estimated as follows:

\[
\text{The production yields of SDs (Y \%)} = \frac{\text{Wtotal} - \text{WF}}{\text{Wtotal}} \times 100 \quad \text{Equ 1}
\]

\[
\text{Drug loading (DL \%)} = \frac{\text{Wtotal} - \text{WF Polymers}}{\text{W Polymers}} \times 100 \quad \text{Equ 2}
\]

Where WF is the analyzed amount of free drug in the supernatant; W total is the theoretical amount of drug that was added; W Polymers is the total amount of Polymers added (total weight of HPMC and EC) [12].

Differential scanning calorimetry (DSC)

The samples (5–10 mg) was thermally sealed in an aluminum pan and heating was carried out at the 5°C / min using a Shimadzu DSC-50, Japan. Nitrogen was used to purge gas through the DSC cell at a flow rate of 50 ml/min over a temperature range of 30-150°C [13].

In vitro drug release study of the drug from the prepared solid dispersion

A weighed quantity of drug solid dispersions from each batch (A, B and C) equivalent to 10 mg of BH were introduced individually into each vessel containing 900 ml of phosphate buffer solution pH 7.4, 37 ± 0.5°C and at a speed of 50 r.p.m in USP basket type apparatus I (Dissolution tester, Pharma test, PTZ, Germany). At a required time intervals, samples were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium maintaining sink conditions throughout the experiment. Samples were analyzed by HPLC as described earlier.

Kinetic analysis of in vitro release study

To study the mechanism of BH release, the cumulative release percent vs time profiles were fitted to different mathematical models as follows:

Zero-order kinetic model: \( \frac{M_t}{M_{\infty}} = KT \)

First-order kinetics model: \( \ln(1 - \frac{M_t}{M_{\infty}}) = KT \)

Higuchi model: \( \frac{M_t}{M_{\infty}} = KT^{1/2} \)

Korsmeyer - Peppas empirical equation model: \( \frac{M_t}{M_{\infty}} = Kt^n \)

Where Mt is the percent of drug cumulatively released at time t, M∞ is the percent of drug cumulative released at the time t = M∞, k is the fraction of drug released at the time point t, k is a kinetic rate constant, and n is the exponent characterizing the mechanism of drug release.

Table 1: The composition of different BH tablets prepared by direct compression method

<table>
<thead>
<tr>
<th>Formulae NO.*</th>
<th>Batch</th>
<th>10% Binder</th>
<th>68% Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>A</td>
<td>PVP K30</td>
<td>Mannitol</td>
</tr>
<tr>
<td>T1</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>A</td>
<td></td>
<td>Lactose</td>
</tr>
<tr>
<td>T2</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>A</td>
<td>PVP K90</td>
<td>Mannitol</td>
</tr>
<tr>
<td>T3</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>A</td>
<td></td>
<td>Lactose</td>
</tr>
<tr>
<td>T4</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All formulae contain; amount of Bisoprolol hemifumarate solid dispersion equivalent to 10mg (10% w/w), Sweeting agent: 1%, Aspartame, and Lubricant; 1% Mg Stearate

Physical evaluation

Tablet Hardness test

The hardness test was carried out using a tablet hardness tester (Hardness Tester, D.R. Schleunger, 6D tablet tester, Germany). Six tablets from each formulation batch were tested randomly, and the average reading was noted. The average hardness is measured in kg/cm² [14].

Tablet friability

The tablets’ friability test was measured according to the United States pharmacopeia (USP 36, 2013) [15]. A sample of tablets corresponding to 6.5 g was placed in the friability tester (Friabilator, Van Seward, PNC, Germany) which was given 100 revolutions (25 r/min for 4 min). The tablets were reweighed for determination of
the percent friability. The friability percent was calculated from the following equation. The friability percent
\[ = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight} \times 100} \text{ Equ} (3) \]

**Tablets wetting time (WT) and tablets wetting absorption ratio (WAR)**

Tablets WT was measured by a modified procedure from that reported by Bi et al. [16]. The procedure was modified as follows: Five circular tissue papers of 10 cm diameter were placed in a petri dish containing an eosin dye solution in water (10 ml of 0.05% w/v). A tablet was carefully placed on the tissue paper. The required time for appearance of the dye solution on the upper surface of tablet [A wetting time (WT)] was recorded using a stopwatch. The water absorption ratio was calculated using the following equation:
\[ \text{WAR} = \frac{W_b - W_a}{W_a} \text{ Equ} (4) \]

Wa and Wb are the weights before and after water absorption, respectively [17].

**Disintegration time**

**Measurement of disintegration time by a modified apparatus:**

Fast disintegrating tablets disintegrate or dissolve in the mouth by saliva. The limited amount of saliva in the mouth and the absence of simulated tablet disintegration test found in US Pharmacopeia made the difficulty to apply the general disintegration test to reflect real conditions. A modified version of simple and novel disintegrating test apparatus developed by Fu et al. was used [18]. The device consisted of a cylindrical vessel in which 10-screen mesh was placed in such way that only 2 ml of disintegrating medium would be placed below the sieve. Disintegration test was carried out at 150 RPM. 2 ml phosphate buffer pH 6.8 was maintained at 37 ± 0.5°C.

**In-vivo oral disintegration time**

A test was performed on six healthy adult volunteers. The time required for the complete disintegration of the tablet when it placed on the tongue was determined by tactile feedback using a stop watch. Permission to carry out this work was obtained from the Institutional Ethics Committee (NODCAR, Giza, Egypt) [19].

**In vitro dissolution study**

Tablet formulae were introduced individually into each vessel containing 900 ml of phosphate buffer solution pH 7.4, 37 ± 0.5°C and at a speed of 50 r.p.m in USP Apparatus II (Dissolution tester, Pharma test, PTZ, Germany) [20]. Aliquots of 3 ml were withdrawn from the dissolution medium at different time interval for 24 hours. The drug content was determined by HPLC method and calculated according to the predetermined yield percent. Each experiment was done in triplicate.

Dissolution efficiency (DE %) is used as the criterion for comparing the effect of polymer concentration on the rate of drug release. DE is defined as the area under the dissolution curve up to the time "t" expressed as percentage of the area of a rectangle described by 100% dissolution at the same time as in equation (5).

Dissolution efficiency (DE %)
\[ \text{DE} = \frac{\int_0^t Y dt}{Y_{100}} \times 100 \text{ Equ} (5) \]

Where \( Y \) is the percent drug release as the function of time, \( t \), \( T \) is the total time of drug release and \( Y_{100} \) is a 100% drug release [21].

**Accelerated stability testing:**

The selected formulae were stored at 60 °C and 75 % relative humidity (maintained using a saturated solution of NaCl) for three weeks [22]. The stored tablets were examined visually for any appearance and/or color changes every week. The tablets' physical evaluation includes the dissolution test was repeated at the end of the storage period as the previously adopted for the fresh tablets. All experiments were repeated three times with three different batches. The results were expressed as the mean ± SD followed by paired t test. Differences are considered to be significant at \( p < 0.05 \). Dissolution profiles of fresh and stored tablets were compared according to the model independent mathematical approach of Moore and Flanner, 1996 [23]. The similarity factor (\( f_2 \)) was calculated according to the following equation:
\[ f_2 = 50 \log \left[ 1 + \left( \frac{n}{10} \right) \sum_{t=1}^{n} \left( \frac{R_t - T_t}{\text{Y}_{	ext{mean}}^{	ext{R}}} \right)^2 \right] \times 100 \text{ Equ} (6) \]

Where \( n \) is the number of sampling points, \( R_t \), and \( T_t \) is the mean percent dissolved of the reference (fresh) and the test (stored) at time \( t \) respectively. \( f_2 \) represents a logarithmic transformation of the sum of squared error of differences between the reference and test products over all time points [24].

**Clinical study**

Thirty patients with primary hypertension (age: 50.9 ± 2.38 years, weight: 83.9 ± 2.81 kg, blood pressure: 165.8 ± 3.90/102.5 ± 1.86 mm Hg, heart rate: 63.6 ± 2.98 min) were included in this study. According to by the Human Ethics Committee of NODCAR (National organization of drug control and research) the study protocol was approved and the institutional guidelines. All the enrolled patients have written informed consent. The test was performed to compare the pharmacokinetic and the pharmacodynamic effect of the best selected formula (Tal1) to commercial oral tablet (5mg Concor ®). The non blind, two treatments, two periods and randomized crossover study were followed. Under this design, half of the subjects were given orally 5mg Concor® twice daily and the other half was given Tal1 buccally once daily (The composition in table 1) with a 7-day inter dose washout periods.

A volume of blood samples (2.0 ml) was drawn through an intravenous catheter and collected at different time intervals in heparinized tubes. Plasma samples analyzed using a modified validated Braza et al. HPLC for determination of BH method after deproteinized by acetonitrile [25]. Blood samples were at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16, 20, and 24 h after oral dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 after buccal administration of formula Tal1 tablet’s group, on day 1 and day 7.

Pharmacokinetic parameters were calculated by a non compartmental method. The area under the serum concentration versus time curve; AUC0–t, AUC0–∞ and mean residence time (MRT) was calculated. Mean residence time (MRT) is the average total time of a given dose spent in the body, which may be calculated as MRT = AUMC/AUC. The Cmax and Tmax were obtained directly from the data. The average steady-state concentration (Cav) is calculated as Cav = AUC0–t / t. Bioavailability is the rate and extent to which the active ingredient is absorbed from a drug product and was performed using the F ratio, where F was the ratio of AUC0–∞ for BH in Tal1 tablets versus market tablets (F=24 for both formulae).

Blood pressures (BPs) were monitored and measured for 24 hours. The difference of the blood pressure induced by each formula expressed as the mean reduction in both DBP and SBP. The data collected at different time intervals and recorded with Mercurial sphygmomanometer. The mean arterial pressure was calculated according to the following equation.
\[ \text{MAP} = \frac{1}{3} (\text{DBP} + \frac{2}{3} \text{SBP}) \text{ Equ} (7) \]

The response to the formulae was expressed by (MAP-AUC) to represent the rate and extent of DBP and SBP reduction.

**Statistical Analysis**

All tests were conducted in triplicates. The results were expressed as the mean ± SD followed by paired t test. One-way Analysis of Variance (ANOVA) was applied to assess the significance of the effect of storage on the physical properties of the tested formulae and the fresh formulae (In all experiments). Two-way Analysis of Variance (ANOVA) was used to assess the significance of the effect of formulation and subject factors on the pharmacodynamic parameters. Guassian’s test for multiple comparisons was then performed to determine the source of difference respectively using SPSS® software version 7.5 (SPSS Inc, Chicago, IL). Differences are considered to be significant at \( p < 0.05 \).
RESULT AND DISCUSSION

HPLC method for the determination of BH

HPLC separation method explains; a mean correlation coefficient ($r^2$) for the calibration curve was over 0.9989. The assay showed acceptable precision and accuracy. The precision ranged from 0.321 to 12.994 (C.V. %) and accuracy ranged from -5.25 to 15.988 (relative error %). The relative standard deviation was lesser than 2%. The LLOQ of the assay was calculated to be 0.05µg/mL. All the results indicated that; the suitability of the HPLC method for the determination of BH in unknown samples.

Percent yield of SDs and drug loading

The production yields of SDs ranged between 93.50 ± 0.39 and 99.20 ± 0.52%. The production yields of SDs for batch A was 93.50 ± 0.39, batch B was 95.987 ± 0.685% while for batch C was 99.20 ± 0.52%. The amount of drugs determined in each SD was 94.56 ± 0.36, 98.72 ± 1.11% and 95.86 ± 0.80 for batches A, B and C respectively. The drug loading (DL %) was for batches A; 29.879 ±0.12%, B; 30.28±0.078% and C; 29.92±0.257%. Statistical analysis shows no significant difference between either batch whether in the production yields or in the drug loading (DL %) or in the drug content.

Drug and Drug-Excipient’s interaction

The thermal curves of BH and BH-SDs along with the physical mixture are shown in Fig 1. The thermal curve of pure BH [Fig 1a] exhibited a sharp endothermic effect. The melting endotherm was with an onset (the point) of 100.53° C and peak at 103.89°C. The enthalpy of fusion (H) was calculated to be -135.75 my/mg. The DSC profile of HPMC, EC, PVP k30 and PVP K90 was showing a large dehydration band in the 50°–120°C temperature range as typical amorphous substances. The thermal curve of the physical mixture; a drug with PVP K90, Mannitol, or Lactose was practically the sum of those of pure components [not shown].

The thermal curve of the BH-SD [Fig 1b, 1c, 1d] for batches A, B, C respectively displayed a reduction of fusion enthalpy and sharpness of the endothermic peak of the drug. That could be attributed to the conversion of most of the crystalline form of the drug to amorphous form [26].

**Fig. 1: Differential scanning calorimetry curves of PVP K30 and K90, HPMC, EC and (a) Bisoprolol hemifumarate, (b) Batch B (c) Batch A (d) Batch C, (e) physical mixtures BH and PVP K30**

However, the combination of BH and PVP K30 shows a change in the endothermic peak of BH [Fig 1e]. In fact, mixing can lower the purity of each component resulting in slightly broader and lower the melting point of the drug (BH). Beside all of that, shift or broadening of peaks may indicate interaction, but not necessarily indicate incompatibility [27].

To be sure there was no interaction between BH and PVP K30; the Infrared spectroscopy (IR) (IR spectrometer, Shimadzu IR-470, Japan) was used. The mixture of BH: PVP K30 (1:1 w/w) reveres that no change in the characteristic peak of the drug, indicating that there was no interaction between BH and PVP K30 [Fig 1f (1,2)].

The characteristic bands (CH-NH- ) 1574, (CH3) 2950-2850, (NH- and OH-) 3200-3600

Notes: Due to the intermolecular H-bonding between branched methyl group (2950-2850) and free –NH and –OH gpe - (3200-3600) gave broadband shifting to (2850-3200) [28].

In vitro drug release and kinetic study of the solid dispersion

The release of the drug from the prepared SD's was calculated as the percentage of BH released according to the predetermined yield value. Ranking the dissolution rate from different batches was A > B > C. In general the average dissolution value of batch A was 90.91% ± 4.32
within 20 hrs ± 0.978, batch B give 93.13% ± 2.17 within 26 hrs ± 2.01 while batch C showed dissolution of 92.43% ± 4.17 within 28 hrs ± 2.74.

It was noted from fig 2 that the increase in the polymer concentration leads to decrease the dissolution rate. The release retardation was a result of increasing in the diffusion path length which drug molecules have to traverse by the effects of ethyl cellulose concentration as well as the negative effect of HPMC on the drug release [29]. HPMC is known to form hydrogels which slowly erodes in aqueous solutions [30]. All the above leads to prolong the dissolution rate of the drug.

The release profiles were evaluated kinetically by zero order, first order, and Higuchi models. The release data obtained according to the determination coefficient ($R^2$) had high linearity ($R^2$: 0.9891 to 0.9956) observed for the Higuchi model. That means both the drug and carrier dissolve at rates proportional to their solubility and diffusion coefficients in the dissolving medium. It mainly applied to solid dispersal systems [31-33].

All the different batches also followed Korsmeyer–Peppas model a simple exponential equation for a drug release fraction ($n<0.5$) [34]. This indicates case, I or simple Fickian diffusion. So, drug release depends on two simultaneous rate processes, the rate of water uptake and the rate of drug diffusion through continuously swelling gel layer surrounding the drug. High polymer content results in a greater amount of gel being formed. This gel and its viscous nature increase the diffusion path length of the drug and furthermore affect the diffusion coefficient of the drug [35]. Hence, the drug release was controlled by a combination of diffusion, polymer relaxation and erosion of the polymeric component.

**Fig. 2: Dissolution profile of the prepared BH-SD batch (A, B and C).**

**Physical characterization of tablets**

All formulae showed acceptable hardness values ranging from 2-5 (USP 36, 2013), except Tc4 and Tb4 Table 2. Taking the binder factor in consideration, significant difference occurred (increasing the hardness value) when changing the binder of smaller particle size with that of larger particle size ($P$-value=0. 158, $SE=0. 156$). The analysis of interaction revealed that; mannitol had the lowest hardness value with a small particle size binder; PVP K30 ($P$-value=0. 138, $SE=0. 062$) (Data not shown). All tablets showed accepted friability value. The friability % was ranging from 0.04-0.56% i.e., Less than 1%

All factors studied had a significant effect on the tablets’ friability ($p<0.05$). Studying the interaction found that only Diluents*PVP K30 interaction had a significant effect on the tablets’ friability ($p > 0.05$) ($P$-value=0. 121, $SE=0. 021$). The friability % of mannitol-PVP K 30 was higher than lactose-PVP K 30 (Data not shown).

**Tablets wetting time (WT) and wetting absorption ratio (WAR)**

Tablets belonging to formulation T1, T3 prepared using mannitol exhibited shorter wetting time than T2, T4 prepared using lactose. In addition tablets formulae prepared with PVP K30 exhibited shorter wetting time than that prepared using PVP K90; Table 2. This could be attributed to the greater number of pores formed in these compressed tablets which resulted from its lower hardness (the reduced tablet porosity retards water penetration and delays or even inhibit the role of the super disintegrant) [36].

Moreover, it was evident that all tablets containing Ac-Di-Sol as a super disintegrant which when moistened, expands and swells to cause rupture and complete the disintegration of the tablet [37]. Hence, the decrease in the wetting time indicated the enhancement of the tablet disintegration. In addition, the high absorption ratio indicating the large amount of water absorbed, which lead to enhance the disintegration of tablets.

**Disintegration time**

**Measurement of disintegration time by a modified apparatus**

The tablets’ disintegration time showed a wide variation from 31 to 83 seconds (Table 2). The data clearly indicate that the disintegration time and hardness values strongly depend on the selected independent variables as shown in Table 2. Tb4 and Tc4 tablet formulations have the longest disintegration time as there was a direct correlation between hardness value and carrier dissolve at rates proportional to their solubility and diffusion coefficients in the dissolving medium. It mainly applied to solid dispersal systems [31-33].

The different batches also followed Korsmeyer–Peppas model a simple exponential equation for a drug release fraction $n<0.5$ [34]. This indicates case, I or simple Fickian diffusion. So, drug release depends on two simultaneous rate processes, the rate of water uptake and the rate of drug diffusion through continuously swelling gel layer surrounding the drug. High polymer content results in a greater amount of gel being formed. This gel and its viscous nature increase the diffusion path length of the drug and furthermore affect the diffusion coefficient of the drug. As a result, a reduction in the drug release rate is obtained [35]. Hence, the drug release was controlled by a combination of diffusion, polymer relaxation and erosion of the polymeric component.
disintegration time. The greatest compact force leads to lower the tablets’ porosity. Luginbuhl and Leuenberger [38] confirmed that the water uptake was the first step in the process of disintegration.

PVP type and diluents’ types had the significant effect on the disintegration time (P<0.05). Not only was there no interaction between the effect of PVP type and the diluents used, but also it had no significant effect on the tablets’ disintegration (Data not shown) (P-value=0.0159, SE=1.844).

Except all formulae have the same type and the same concentration of the disintegrant (5% Ac Di Sol) but each formula has different disintegration time. This was explained by the previously reported fact; the rate at which the binder dissolved is the main factor in the tablets’ disintegration. It was distributed across the particle surface [39].

Tablets formulae T1 and T2 containing PVP K30 as a binder have disintegration times lesser than one minute. PVP K30 has the smallest particle size in addition; the uneven surface of PVP with a folded structure increases the area subjected to the disintegration media which led to decrease the disintegration time [40].

**In-vivo oral disintegration time**

Only seven formulae namely Ta1, Tb1, Tc1, Tb2, Ta3 and Tb4 showed disintegration times less than three minutes; 67, 89, 128, 146, 173, 180 and 163 seconds respectively (European Pharmacopoeia, 2002, adopted the term orodispensible tablet as a tablet to be placed in the mouth and disintegrated in lesser than 3 min) [41].

From all previous study tablets formulae Ta1, Tb1, Tc1, Tb2, Ta3, and Tb3 have the best physical characters of all previous evaluation tests. Tb4 was excluded as it has unaccepted hardness value hence; it was not used for the following evaluation tests.

**In vitro dissolution study**

Dissolution samples were analyzed by HPLC method as previously described. The best selected formulae were compared in this respect with the release of the market product (10 mg Concor® tablets). The values were calculated as the percentage of BH dissolve according to the predetermined yield value. The market product (10 mg Concor® tablets) shows the fastest dissolution rate completed in the first hours. It gave 98.41% within 60 minutes; Fig 3A.

<p>| Table 2: Physical evaluation of the stored and the fresh tablets |
|-----------------------------|-------------|-------------|-------------|-------------|-------------|
| <strong>The fresh tablets</strong>       |             |             |             |             |             |</p>
<table>
<thead>
<tr>
<th>F</th>
<th>H. *</th>
<th>%F*</th>
<th>Dt**</th>
<th>Dt***</th>
<th>WT#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta1</td>
<td>3.1±0.03</td>
<td>0.52</td>
<td>34±0.23</td>
<td>67±0.91</td>
<td>10.2±0.44</td>
</tr>
<tr>
<td>Tb1</td>
<td>3.6±0.25</td>
<td>0.04</td>
<td>36±0.24</td>
<td>89±0.15</td>
<td>10.5±0.71</td>
</tr>
<tr>
<td>Tc1</td>
<td>3.8±0.09</td>
<td>0.23</td>
<td>31±0.24</td>
<td>128±0.56</td>
<td>11.5±0.71</td>
</tr>
<tr>
<td>Ta2</td>
<td>3.95±0.58</td>
<td>0.50</td>
<td>35±0.06</td>
<td>206±0.02</td>
<td>31.3±0.14</td>
</tr>
<tr>
<td>Tb2</td>
<td>4.0±0.07</td>
<td>0.56</td>
<td>45±0.08</td>
<td>146±0.03</td>
<td>29.1±0.48</td>
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<tr>
<td>Tc2</td>
<td>4.3±0.57</td>
<td>0.07</td>
<td>53±0.06</td>
<td>217±0.01</td>
<td>28±0.048</td>
</tr>
<tr>
<td>Ta3</td>
<td>4.8±0.04</td>
<td>0.073</td>
<td>68±0.07</td>
<td>173±0.45</td>
<td>19.3±0.14</td>
</tr>
<tr>
<td>Tb3</td>
<td>4.9±0.59</td>
<td>0.11</td>
<td>69±0.09</td>
<td>180±0.25</td>
<td>17±0.14</td>
</tr>
<tr>
<td>Tc3</td>
<td>4.9±0.9</td>
<td>0.04</td>
<td>71±0.59</td>
<td>199±0.09</td>
<td>18 ±0.84</td>
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<tr>
<td>Ta4</td>
<td>4.87±0.01</td>
<td>0.12</td>
<td>65±0.06</td>
<td>206±0.41</td>
<td>85±0.048</td>
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<tr>
<td>Tb4</td>
<td>5.1±0.025</td>
<td>0.01</td>
<td>73±0.04</td>
<td>163±0.03</td>
<td>180±0.39</td>
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<tr>
<td>Tc4</td>
<td>5.9±0.96</td>
<td>0.03</td>
<td>83±0.12</td>
<td>301±0.4</td>
<td>185±0.39</td>
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<td><strong>The stored tablets</strong></td>
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<td></td>
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<tr>
<td>F</td>
<td>H. *</td>
<td>%F*</td>
<td>Dt**</td>
<td>Dt***</td>
<td>WT#</td>
</tr>
<tr>
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<td>-----</td>
<td>-----</td>
<td>------</td>
<td>-------</td>
<td>-----</td>
</tr>
<tr>
<td>Tb2</td>
<td>2.1±0.14</td>
<td>0.93</td>
<td>170±0.45</td>
<td>173±0.11</td>
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<tr>
<td>Ta1</td>
<td>2.0±0.11</td>
<td>0.12</td>
<td>68±1.21</td>
<td>69±0.23</td>
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<tr>
<td>Tb1</td>
<td>2.5±0.43</td>
<td>0.87</td>
<td>74±1.12</td>
<td>74±0.91</td>
<td></td>
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</tbody>
</table>

F: Formula number H. Hardness (Kg/cm2) ± SD %F*: %Friability Dt**: Measurement of disintegration time by a modified apparatus SD Dt***: In-vivo oral disintegration times SD #WT: Wetting Time ##WAR: wetting absorption ratio.

Fig. 3A: Dissolution profile of the market product 5mg Concor® for 60 minutes.

Ranking the dissolution rate from different formulae was Ta1>Ta3> Tb1> Tb2>Tb3> Tc1. In general the average dissolution value of batch A was 98.98% ± 5.47 within 18 hrs ± 1.078, batch B gave 96.63% ± 6.47 within 20 hrs ± 0.47 while batch C showed dissolution of 98.63% ± 4.17 within 23 hrs ± 1.84.

Statistical analysis of the results when comparing Ta1 with Tb1 and Ta3 with Tb3 indicate that increase in the amount of HPMC, it seems that there is no change in the release pattern (P-value=0.207, SE=0.0967). But when compare Ta1, Tb1 and Tc1 with each other indicate that increase in the amount of EC, it seems that has a significant effect in retard the release pattern.

The reduction in the dissolution rate was in the same pattern as previously reported in the in vitro release study of the solid dispersion. Comparing the dissolution value of Tb1 and Tb2 found that; there was a significant effect on mixing BH with anhydrous lactose. The latter showed the dissolution rate profile lower than the dissolution rate profile of the formula containing mannitol; 55.81%, 64.52% for Tb1 and Tb2 in the first 12 hrs respectively, Fig 3B.

The standard deviation was emitted to increase the clarity of the figure.

Comparing the dissolution result of (Ta1, Tb1, and Tb2) with (Ta3 and Tb3) found that; PVP K30 has a significant effect on the dissolution rate. The presence of PVP K30 in Ta1, Tb1, and Tb2 can probably be increased the wetability of the drugs as, PVP K30 causes decreased the interfacing tension between the drug and the dissolution media [42].
DE was used to compare the dissolution rate and the polymer effect on it. The DE value was 93.86%, 83.58%, 63.97%, 72.25%, and 70.98% for Ta1, Tb1, Tc1, Tb2, Ta3, and Tb3 respectively. The DE value for Ta1 containing 1:1 of HPMC: EC ratio is 93.86%. Whereas this value decreased to 77.96% and 63.97% for Tb1, Tb2 (average), and Tc1 containing 1:2 and 2:1 ratio of HPMC: EC, respectively. That improving there is a retardation in the drug release rate by increasing the amount of EC and there was no significant change while increasing HPMC amount (P-value=0.0135, SE=0.635).

From the previous study tablets formulae Ta1, Tb1 and Tb2 were selected as they have the highest DE. Ta3 and Tb3 were excluded as it contains PVP K90 which has a negative effect on the dissolution rate and Tc1 have the lowest DE (< 70%). Hence, Ta1, Tb1 and Tb2 were used for the following evaluation tests.

To be more precise; the dissolution result of the selected prepared tablets (Ta1, Tb1 and Tb2) compared with the dissolution result of the BH-SD to evaluate if the tablet compression has any effect on the retardation of drug release. From Fig. 3C, it is clear that a slightly faster release was observed during the first hour with tablet compared with that of uncompressed SD-BH. However, a significant difference was not observed (p > 0.05) in the release patterns. It means the dissolution rate mainly depend on the composition of the SD.

By studying the mechanism of drug release from each formula, the dissolution data of batches were fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas as earlier reported.

The slight increase in the dissolution rate could be due to the effect of the super disintegrant which enables the minimal rupture/fracture of solid dispersion particle. In addition, the effect of the water soluble diluents (mannitol and lactose) and PVP K30 enhance the wetting of the SD particles [43].

The standard deviation was emitted to increase the clarity of the figure.
The in vitro release profiles of drug from all the formulations could be best expressed by Higuchi's equation (cumulative percentage) as the correlation coefficient values ($R^2$) had high linearity ($R^2$: 0.990 to 0.999). It was found that different formulae had $n<0.5$; this indicates case I or simple Fickian diffusion (Data not shown). Hence, diffusion was controlled by a combination of diffusion and polymer relaxation.

**Accelerated stability testing**

None of the stored formulae (Ta1, Tb1 and Tb2) showed any change in color or appearance throughout the storage period. On the other hand, some tablets of Tb2 showed faint discoloration by the 2nd week. The characters of the stored tablets are summarized in the table 2. Formula Tb2 contains a high percentage of lactose which undergo a non-enzymatic browning reaction with amines (active group in BH) generally known as the Maillard reaction. That reaction resulted in the discoloration and the brown spots at the end of the storage period [44].

According to Moore and Flanner equation; the dissolution profiles to be considered similar, the value of the similarity factor ($f_2$) should be as close as possible to 100 (range from 50 to 100, corresponds to 10% and 0% differences, respectively). The computed ($f_2$) values were 75.27%, 69.05% and 56.22% for Ta1, Tb1 and Tb2, respectively, indicating that the dissolution profiles of fresh and stored tablets could be considered similar, Fig 4. The highest ($f_2$) value was in case of Ta1 and Tb1. It was indicated the higher the similarity between the dissolution profiles of fresh and stored tablets of these formulae.

![Graph](image)

**Fig. 4:** The dissolution profile of the fresh formulae compared to the dissolution profile of the stored formulae (Ta1, Tb1 and Tb2).

![Graph](image)

**Fig. 5:** The mean serum concentration-time curves after single and multiple doses of: A: BH oral tablet 5mg Concor® (n = 30). B: BH extended-release tablet Ta1 (n = 30).
Physical evaluation of formulae Tb1 and Ta1 showed a marked decrease in the mechanical strength of formula Tb1 compared to Ta1 (hardness value). Although the mechanical strength of formula Tb1 was less than fresh one but according to ICH guidelines (ICH Q1, Q6a) some physical changes in attributes may be expected under accelerated conditions [11]. Furthermore, statistical analysis approved that; all results (hardness, friability and disintegration time) had no significant difference in the stored formulae Ta1 compared to fresh formula Ta1. Hence, this formula was physically stable after storage (The level of significance of rejection is more than 0.25) [11].

Based on all results of storage at 60 °C and 75 % RH for three weeks, and the other physical characters; formula Ta1 was the best selected formula.

Clinical study
Mean serum concentration-time curves of BH formulations on day 1 and day 7 are shown in Figures 5 (a and b), and the pharmacokinetic properties are summarized in Table 3. While the mean values for the area under the mean reduction of MAP-time curve AUC (0-24) was illustrated in Fig 6.

The results of the statistical analysis revealed that the formulation had a significant effect on $T_{max}$, AUC (0-24) and AUC (0-∞) of BH at p < 0.05 (FS; 17 n = 6.469, 43.890 and 242) respectively. Based on these results, it was evident that the formulation exhibited the most significant effect on AUC (0-24). On the other hand, there was no significant difference between the subjects for all the tested parameters, CPmax and Cav. Multiple comparisons using Guassian’s test revealed that AUC (0-24) was extremely differed significantly with the highest value for Ta1.

The mean values of the area under the mean reduction of MAP-time curves AUC (0-24) were 17.39 ± 1.92 and 39.479 ± 4.62 after the oral administration of the market product 5 mg Concor® and the buccal administration of Ta1 to the thirty subjects, respectively at p < 0.05 (P-value = 0.35). Based on these results, it was evident that the formula Ta1 exhibited a significant difference effect in reduction of the mean arterial pressure.

The formula Ta1 was developed to decrease the frequency of the dose administration and maintain the peak serum concentrations after drug administration and maintain the reduction of the mean arterial pressure. The peak concentration of Ta1 was delayed. In addition, there was less fluctuation compared to market formulation.

Table 3: Pharmacokinetic parameters of BH of two BH formulations after single dose and multiple doses at the end of 7- days (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Test (Ta1 tablet)</th>
<th>Reference (5mg Concor®)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>1.25 ± 39.0</td>
<td>1.3 ± 271</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>6.258 ± 1.34</td>
<td>2.547 ± 0.38</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>23.216 ± 2.28</td>
<td>9.95104 × 0.44</td>
</tr>
<tr>
<td>AUC (0-24) (ng·h/mL)</td>
<td>24.628 ± 1.56</td>
<td>18.7465 ± 2.776</td>
</tr>
<tr>
<td>F (%)</td>
<td>49.10886</td>
<td>28.16401</td>
</tr>
<tr>
<td>AUC∞ (ng·h/mL)</td>
<td>174.68 ± 23.9</td>
<td></td>
</tr>
<tr>
<td>F (%)</td>
<td>1.219 ± 29.7</td>
<td>1.14 ± 255</td>
</tr>
<tr>
<td>Multiple dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{max}$ (ng/mL)</td>
<td>2.60 ± 0.87</td>
<td>1.88 ± 0.36</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>20.57 ± 2.40</td>
<td>10.43 ± 0.30</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>32.2 ± 3.1</td>
<td>27.985 ± 3.6</td>
</tr>
<tr>
<td>AUC (0-24) (ng·h/mL)</td>
<td>53 ± 6.1</td>
<td>33.5 ± 4.4</td>
</tr>
<tr>
<td>Cav(ng/ml)</td>
<td>1.34 ± 0.77</td>
<td>1.16 ± 1.42</td>
</tr>
<tr>
<td>F (%)</td>
<td>158.3± 24.1</td>
<td></td>
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</table>

CONCLUSION
The present study indicates; the suggested buccal tablet (Ta1) which contains solid dispersions’ 10 mg BH using HPMC and EC in 1:1 ratio showed an extended effect in lowering the blood pressure than commercial oral tablets. The Ta1 formulation of bisoprolol hemifumurate was developed to decrease the frequency of dose. In addition, it considered a promising dosage form easy for handling of elderly patients suffering from hypertension once every 24 hr. It can be taken with no access of water as; it is orodispersible tablets with short disintegrating time. The significant findings presented here encourage further studies.

REFERENCES
10. [Murpah, 1875 #3086]
42. Sammour OA, Hammad MA, Megrab NA, Zidan AS. Formulation and optimization of mouth dissolve tablets containing rofecoxib solid dispersion. AAPS PharmSciTech 2006;Jun1;6(7):E55.