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Original article

Impacts of storage conditions on the dissolution performance of commercial metronidazole tablets available in Saudi Arabia

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ABSTRACT

This study aimed to investigate the impact of storage conditions on the dissolution performance of commercial metronidazole (MTZ) tablets available in Saudi Arabia; these were coded as the reference and Test A, Test B, and Test C products. Moreover, the hardness and the disintegration time were measured. The UV spectrophotometrically analytical technique was utilized to quantify MTZ. All the control tablets, which were tested upon receipt, met the USP requirement as not less than 85 % of the labeled amount of MTZ was dissolved in 60 min. The MTZ reference released 91.79 % ± 1.23 after 60 min, while the products A, B, and C released 87.96 % ± 2.60, 93.26 % ± 2.01, and 88.61 % ± 2.04, respectively. The different dissolution parameters calculated for all the control tablets showed that the MTZ products A and B had optimal dissolution performances and were considered similar to the reference product. The product C showed a significantly reduced dissolution performance and was considered different from the reference. The in vitro dissolution of the MTZ tablets stored at 40oC ± 2 oC/75 % RH ± 5 % for 6 months indicated that the tablets maintained compliance with the USP requirement. The MTZ reference released 89.36 % ± 3.64 after 60 min, while the products A, B, and C released 95.79 % ± 3.91, 88.52 % ± 2.52, and 87.79 % ± 5.04, respectively. However, a slight reduction in the percentage released after 30 min (% DE30) and a slight increase in the mean dissolution time (MDT) were observed during the first 3 months of storage under stressed conditions. These changes were more obvious after 6 months of storage under the same conditions. Furthermore, in vitro dissolution of the product C stored at 40oC ± 2 oC/75 % RH ± 5 % for 3 months with further protection against high humidity revealed an improvement in the dissolution parameters due to the similar protective effects exerted by the two packaging forms. Furthermore, the study shows that storage conditions such as humidity and temperature affect in vitro dissolution of MTZ marketed tablets which may have an impact on efficiency and patient safety.

1. Introduction

Metronidazole (MTZ), or 2-(2-methyl-5-nitroimidazol-1-yl) ethanol (Fig. 1), is an antiprotozoal, antibacterial, and amebicidal drug; it belongs to the group of nitroimidazole antibiotics, which are highly potent and able to cure infectious diseases. It is one of the mainstay drugs in the World Health Organization's Essential Medicines List for the treatment of various bacterial and parasitic infections (WHO, 2021). The medication is generally used to treat anaerobic bacterial infections, such as bacterial vaginosis, amoebiasis, and infections of the stomach or intestine. It is used in combination therapy with other medications to eradicate Helicobacter pylori, which causes peptic ulcer disease (Bhangale and Wagh, 2017). MTZ diffuses into the organism, inhibits protein synthesis by interacting with DNA, and causes a loss of helical DNA structure and strand breakage. Therefore, it causes cell death in susceptible organisms (Weir and Le, 2022).

MTZ is available in various dosage forms, including tablets, capsules, creams, gels, and injections. Among these forms, the tablet form is the

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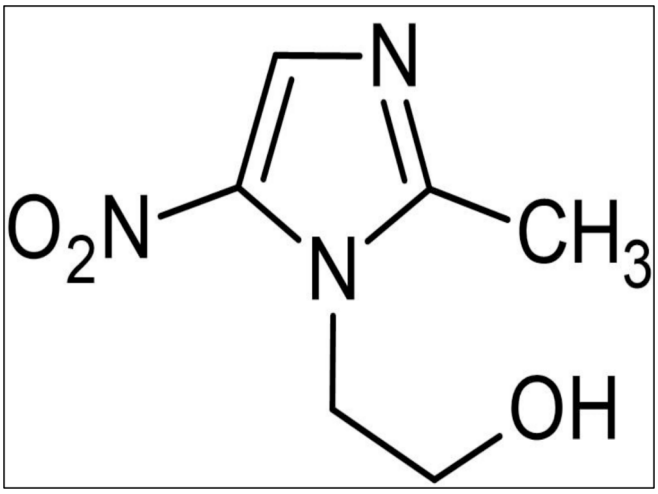

Fig. 1. Chemical structure of MTZ (molecular weight = 171.15 g/mol).

most commonly used and the most widely available. According to the Biopharmaceutics Classification System (BCS), MTZ is classified as a class 1 drug and is characterized by high water solubility and high permeability. Thus, drug absorption depends on the ability of the drug to go into solution/dissolution after oral administration and to then permeate the biological membrane of the gastrointestinal tract. Therefore, the dissolution process is critical in the prediction of the in vivo behavior of the drug (Ilomuanya et al., 2015). MTZ is rapidly absorbed after oral administration, with a bioavailability approximating 100 % (Aleanizy et al., 2017). The dissolution of MTZ tablets is an important factor that affects the bioavailability and efficacy of the medication (Li et al., 2021). Several studies have investigated the dissolution performance of various MTZ commercial tablets (Löbenberg et al., 2012; Reddy et al., 2014; TIlomuanya et al., 2015).

Since metronidazole belongs to the BCS Class I drugs, it qualifies for a biowaiver which means that bioequivalence and in vivo bioavailability studies can be replaced by performing in vitro dissolution studies to compare a "test" product to a reference product. Biowaivers ensure good quality of generic products at reduced cost. Significant variations in the dissolution profile of immediate release metronidazole tablets can impact the rate and extent of drug absorption and hence, the therapeutic effect. Therefore, several guidance documents were issued by regulatory authorities to describe the requirements that are recommended to determine the in vitro bioequivalence of immediate release oral dosage forms using dissolution studies (Reddy et al., 2014).

The dissolution rate of tablets is affected by several factors related to the drug (such as the solubility, particle size, and crystalline state), the excipients used in formulation of tablets (e.g., binder, and disintegrants), and the conditions of the gastrointestinal tract (Hasan et al., 2017). In addition, processing factors such as compression force, and formulation technique affect the final tablet properties and dissolution. Excessive compression force applied during tablet manufacture may lead to a reduction in pore size and increased tablet hardness, resulting in slower drug release (Patere et al., 2015).

The resulting dissolution profiles can be compared using model-dependent and model-independent approaches. The dissolution profiles may be compared in a model-dependent manner to the models of drug releases, i.e., the zero-order model, first-order model, Higuchi model, and Hixson–Crowell model. On the other hand, the model-independent approach utilizes the difference (f_1) and similarity (f_2) factors (fit factors), the dissolution efficiency (DE), and the mean dissolution time (MDT) to compare different dissolution profiles. The difference (f_1) and similarity (f_2) factors may confirm the pharmaceutical equivalence of two dissolution profiles. In addition, for analysis and comparison of the various dissolution profiles, it is possible to use the DE parameter (Anderson et al., 1998). The fit factors, difference (f_1) and similarity (f_2), can also be calculated using the equations reported in (Anderson et al., 1998).

There are some of the quantitative analytical techniques which are used for quality control of pharmaceutical products. Several techniques like ultraviolet/visible spectrophotometry, (Oliveira et al., 2020; Madani., 2024), high-performance liquid chromatography (Anderson et al., 2021; Mirakor et al., 2008), and fluorimetry (Abdel and Shaalan., 2010; Walash et al., 2009) are the popular instrumental technique used for the analysis of pharmaceuticals.

This study was designed to assess and compare the effect of accelerated storage situations (high relative humidity and temperature), over a time period of 6 months, on the stability of the in vitro dissolution performance of four commercial products of MTZ tablets available on the Saudi market (coded as reference, Test A, Test B, and Test C) using USP dissolution apparatus 1. Moreover, the hardness and disintegration time were examined. The study also aimed to investigate the protective effect of different packaging materials such as polyethylene bags with zippers or child-plastic type resistant containers on the dissolution profiles of a selected commercial tablet product (Test C) stored under stressed conditions for 3 months.

2. Materials and methods

2.1. Materials

Authentic MTZ powder was helpfully provided by Riyadh Pharma (Riyadh, Saudi Arabia); hydrochloric acid was also acquired (Sigma–Aldrich, St Louis, MO, USA). Different commercial products of the MTZ tablets were used; the reference (France, batch number 2R464, 500 mg), Test A (Saudi Arabia, batch number 22DE57, 500 mg), Test B (Saudi Arabia, batch number 2KV770, 500 mg), and Test C (Saudi Arabia, batch number 3942, 500 mg) were purchased from community pharmacies in Riyadh, Saudi Arabia. All the other chemicals and reagents used were of analytical grade. The information of different commercial products of MTZ tablets used in this study are presented in Table 1.

2.2. Construction of the standard calibration curve of MTZ in 0.1 moL L^{-1} HCl

A stock solution of MTZ (2 mg mL^{-1}) was prepared in 0.1 moL L^{-1} HCl. The stock solution was prepared as a diluted MTZ solution of 100 μg mL^{-1} in 0.1 moL L^{-1} HCl. Thereafter, a series dilution of the modified MTZ solution in 0.1 moL L^{-1} HCl was performed to obtain various concentrations ranging from 4 to 20 μg mL-1. The absorbance of these dilutions was established spectrophotometrically (Libra S22 UV/Visible Spectrophotometer, Cambridge/United Kingdom) at λ_{max} 277 nm (Das and Dhua, 2014) using 0.1 moL L^{-1} HCl as a reference. All the samples were analyzed in triplicate, and the results are presented as an average ± SD value.

2.3. Storage of different MTZ tablet products under accelerated conditions (40 °C ± 2 °C/75 % RH ± 5 %)

The standard storage conditions for an accelerated stability study of a pharmaceutical product are 40 °C ± 2 °C/75 % RH ± 5 % for 6 months, according to the International Conference on Harmonisation, Guidelines (ICH, 2003). Based on these regulations, the study was planned with the intention to store samples of the MTZ tablets under the same accelerated conditions for 6 months. For the purpose of studying the effect of elevated temperature and humidity conditions on the dissolution performance of the MTZ tablets, the samples of each commercial tablet product were stored in their original blister packaging in a desiccator containing a saturated salt solution of sodium chloride to produce the desirable relative humidity (75 % RH ± 5 %) (Young et al., 2003). The desiccator was then placed inside an oven adjusted to 40 °C

Table 1
Information of different commercial products of MTZ tablets used in this study.

Product Code	Country of origin	Batch number	Manuf. date	Exp. date	Strength	Excipients
Reference	France	2R464	Mar-22	Feb-25	500 mg	Wheat starch, povidone K30, magnesium stearate, hypromellose, macrogol 20000.
Test A	Saudi Arabia	22DE57	Jul-22	Jul-25	500 mg	Maize starch, Lactose (200 MESH), Microcrystalline cellulose (PH 101), Croscarmellose sodium, Colloidal anhydrous silica, Stearic Acid, Purified talc. Coating material: Opadry II White OY-L-28900
Test B	Saudi Arabia	2KV770	Feb-22	Feb-25	500 mg	Microcrystalline cellulose, povidone, starch, kollidon, silicon dioxide, magnesium stearate, methyl cellulose, polyethylene glycol, simethicone, talc and E171.
Test C	Saudi Arabia	3942	Nov-21	Nov-24	500 mg	Information for excipients was not available

± 2 °C. The stored tablets were removed after 0.5, 1, 2, 3, and 6 months and tested for dissolution behavior. The results of the different dissolution tests performed on the stored tablets were compared to the results obtained from the initial or control tablets tested immediately following their receipt. Also, this study was designed to evaluate the effect of different packaging materials on the dissolution performance of MTZ tablets stored under accelerated conditions. For this purpose, samples of the original blister packaging of the Test C commercial tablets were inserted into either polyethylene bags with zippers (160 x 100 x 0.114 mm, Hebei Anda Packaging Co., Ltd., China) or child-plastic type (high density polyethylene) resistant containers; otherwise, they were kept as they were, without further packaging. The differently packed tablets were stored at 40 °C ± 2 °C/75 % RH ± 5 % for 3 months. These specially packed tablets were removed after 3 months of storage and tested for dissolution behavior. The dissolution profiles obtained for the specially packed Test C commercial tablets were compared to those obtained for the corresponding tablets stored in their original blister packaging, without additional packaging, for 3 months under the same accelerated conditions.

2.4. Hardness (Crushing Strength)

Tablet hardness is usually measured to assess the structural integrity and the breaking point of a tablet upon handling, storage, or transportation. The crushing strength of 5 tablets from each brand was determined using a hardness tester (PTB 311E, Test Pharma, Hainburg/Germany). The force required to crush each tablet was measured in kg/cm^2, and the results are expressed as the mean ± SD. The hardness limit described in the USP (USP 41, 2018) falls within the range of 5–10 kg/cm^2.

2.5. Disintegration time

Six tablets from each brand were tested for disintegration in 0.1 moL L^{-1} HCl maintained at 37C^o ± 1C^o, using a tablet disintegration tester (PTZ-S Single Basket Tablet Disintegration Tester, Test Pharma, Hainburg/Germany). The time required for the entire tablet to disintegrate completely and disappear from the surface of the mesh screen was recorded as the disintegration time. According to the USP specifications, if one tablet does not disintegrate within 30 min, the test is repeated on a further 12 tablets; then at least 16 tablets must be completely destroyed in less than 30 min. The disintegration time was measured for 3 sets of tablets from each commercial product, and the results are expressed as the mean ± SD. According to the USP specifications, film-coated tablets should disintegrate within 30 min (USP 41, 2018).

2.6. In vitro dissolution studies

In vitro dissolution studies of the control and the stored commercial MTZ tablets were performed after 0.5, 1, 2, 3, and 6 months of storage using USP dissolution apparatus 1 (Pharma Test, PT – DT70, Hainburg, Germany) according to the requirements specified in the United States Pharmacopeia (USP 41, 2018) for MTZ tablets. The dissolution medium was composed of 900 mL of 0.1 moL L^{-1} HCl maintained at 37 °C ± 0.5 °C, and the rotational speed was adjusted to 100 rpm. Samples of 5 mL were removed at predetermined time intervals, filtered through a 0.45 μm syringe filter, appropriately diluted, and analyzed spectrophotometrically for the medication at 277 nm, against a blank composed of a fresh dissolution medium. The drawn specimens were replaced with a similar volume of fresh dissolution medium that was kept at 37 °C. Six tablets from each product were used for the dissolution studies, and the results are presented as the mean ± SD. The dissolution profiles were constructed by plotting the cumulative % drug released against time.

2.7. Analysis of data

In order to compare the dissolution performances of the different MTZ tablet products, the dissolution parameters, such as the dissolution efficiency after 30 min (% DE_{30}) and the mean dissolution time (MDT), were calculated using DDSolver (MS Excel add inn). Also, the similarity (f_2) and difference (f_1) factors (or fit factors) were estimated using DDSolver to confirm the equivalence of different dissolution profiles. The stability of the dissolution profiles was estimated by comparing the dissolution parameters obtained from the stored tablets to those obtained for the control or initial tablets.

2.8. Statistical analysis

The different dissolution parameters were statistically compared using one-way analysis of variance (ANOVA) with a *t*-test. Statistical differences yielding $p < 0.05$ were considered significant.

3. Results

3.1. The standard calibration curve of MTZ in 0.1 moL L^{-1} HCl solution

The standard calibration curve of pure MTZ in the 0.1 moL L^{-1} HCl solutions was constructed as shown in Fig. 2. Different concentrations of the pure medicinal product were prepared in 0.1 moL L^{-1} HCl, which ranged between 4 and 20 μg mL^{-1}, and the absorbances were determined spectrophotometrically at 277 nm (Das and Dhua, 2014). The resulting standard calibration curve was linear during the examined concentration ranges and obeyed the Beer–Lambert principle with a correlation coefficient (R^2) of 0.9993.

3.2. In vitro dissolution of commercial MTZ tablets obtained from different manufacturers

The in vitro dissolution of the different commercial brands of MTZ tablets was carried out in 0.1 moL L^{-1} HCl. The dissolution profiles obtained for the control tablets (the tablets evaluated upon receipt) are presented in Fig. 3. It can be seen that all the MTZ commercial tablets showed a satisfactory dissolution behavior that complied with the USP requirement as not less than 85 % of the labeled amount of MTZ was dissolved in 60 min. The MTZ reference tablets released 91.79 % ± 1.23

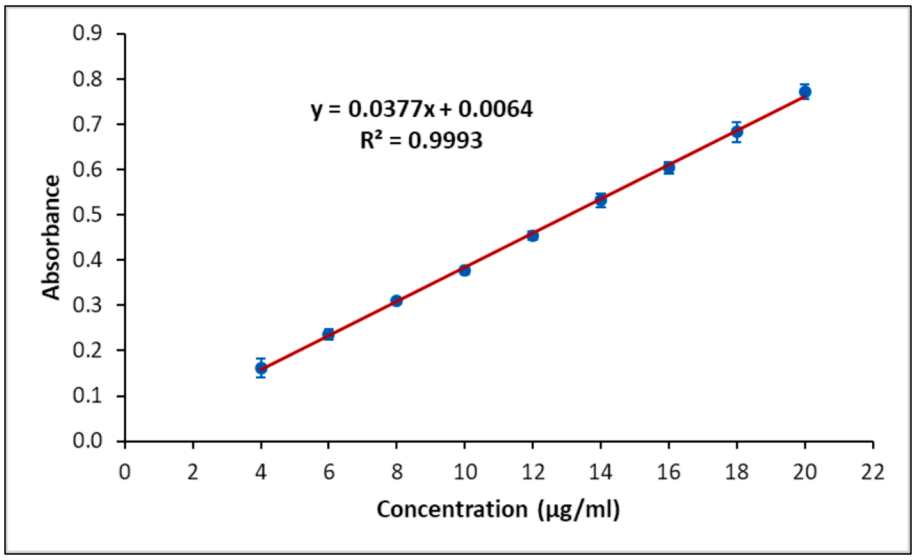

Fig. 2. Standard calibration curve of MTZ constructed in 0.1 moL L^{-1} HCl and assayed at λ_{max} 277 nm.

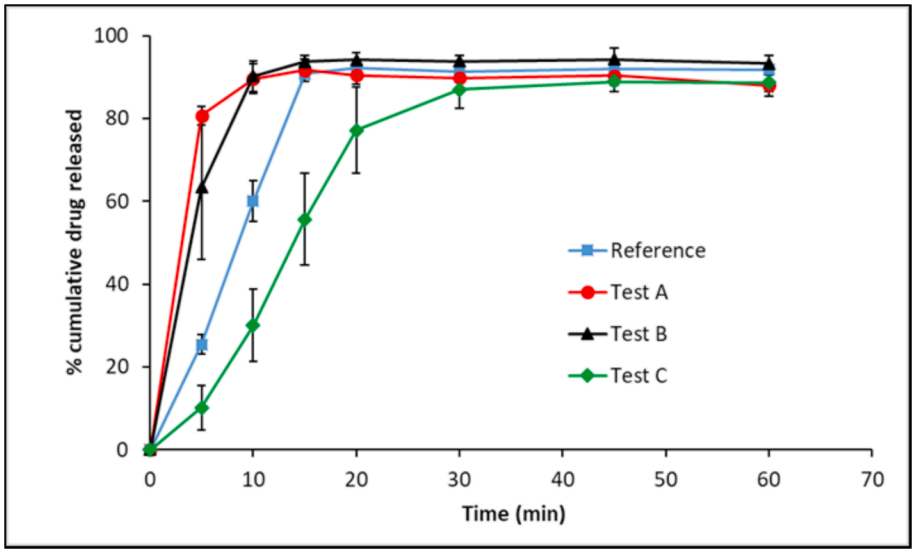

Fig. 3. In vitro dissolution profiles of MTZ tablets (mean ± SD, n = 6) obtained in 0.1 moL L^{-1} HCl for different commercial brands.

% of their content after 60 min, while the test products A, B, and C released 87.96 % ± 2.60 %, 93.26 % ± 2.01 %, and 88.61 % ± 2.04 % of their contents, respectively, in the same time interval.

Because a comparison of the various dissolution profiles built on a particular point measurement may not adequately distinguish the dissolution process (Podczeck, 1993), different dissolution parameters for example the mean dissolution time (MDT) and the dissolution efficiency after 30 min (% DE_{30}), were evaluated for the different brands of tablets handled in this investigation. The dissolution efficiency (DE) can be defined as the area under the dissolution curve up to a certain time, t; it is expressed as a percentage of the area of the rectangle described by 100 % dissolution in the same time period. The value of DE can be calculated via the following equation:$DE = \frac{\int_{t1}^{t2} y.dt}{y_{100}.(t_2-t_1)} \times 100\%$, where y is the percentage of dissolved product, t_1 and t_2 are the time points, and y_{100} is the maximum dissolution percentage. The parameter of the mean dissolution time (MDT) can be estimated via the quantity of drug dissolved in the dissolution equation:$MDT = \frac{\Sigma[t_i.\Delta Q_i]}{Q_\infty}$, where t_i is an interval of the sampling time, ΔQ_i is the amount of drug dissolved in interval t_i, and Q_∞ is the maximum amount of drug dissolved (Anderson et al., 1998). In addition, the difference (f_1) and similarity (f_2) factors were calculated to assess the difference or similarity of the different brands of MTZ tablets (Test A, Test B, and Test C) relative to the innovator or reference brand. All of these parameters are presented in Table 2.

It is clear that in comparison with the reference product, the MTZ test products A and B achieved optimal dissolution. This was shown by their considerably greater values of % DE_{30} and their significantly lower values of MDT ($p < 0.05$) when matched to the corresponding values attained for the reference tablets (Table 2). On the other hand, the MTZ test product C displayed a significantly lower value of % DE_{30} and a significantly higher value of MDT ($p < 0.05$) when compared with the values detected for the reference tablets (Table 2).

The comparison of the dissolution profiles based on the calculation of the fit factors for all the MTZ test products, A, B, and C, revealed $f1$ values of 17.38, 14.56, and 19.52, respectively, and f_2 values of 32.63, 38.17, and 35.58, respectively. Values of $f1$ that approach or exceed 15 and values of f_2 that are below 50 indicate dissimilarity (or difference) between the dissolution profiles (Anderson et al., 1998).

Table 2
Hardness, disintegration time, mean dissolution time (MDT), mean dissolution efficiency (%DE_{30}), similarity factor (f_2), and difference factor (f_1) calculated for different commercial MTZ tablet products.

Product	Hardness (Kg/cm^2) (mean ± SD)	Disintegration time (mean ± SD)	% DE_{30} (mean ± SD)	MDT (mean ± SD)	f_1	f_2
Reference	9.59 ± 0.61	7.25 ± 0.83	67.63 ± 1.68	7.90 ± 1.03	––	––
Test A	10.27 ± 0.97	1.31 ± 0.33*	81.23 ± 1.43*	1.73 ± 1.03*	17.38	32.63
Test B	12.54 ± 0.52*	5.75 ± 0.33*	80.38 ± 2.85*	3.95 ± 1.01*	14.56	38.17
Test C	39.46 ± 0.43*	13.26 ± 0.95*	49.75 ± 4.09*	13.25 ± 2.71*	19.52	35.58

* Significant difference at $p < 0.05$.

3.3. *In vitro dissolution studies of different commercial brands of MTZ stored at high temperatures and relative humidity*

Different commercial MTZ tablet products were subjected to accelerated physical stability studies through storage at 40 °C ± 2 °C/75 % RH ± 5 % for 6 months. After 0.5, 1, 2, 3, and 6 months of storage, the MTZ tablets were tested to evaluate their dissolution properties compared with those obtained from the control tablets for the relevant product. It can be concluded that all the MTZ commercial tablets after storage at each time point presented a satisfactory dissolution behavior that fulfilled with the USP requirement as not less than 85 % of the labeled amount of MTZ was dissolved in 60 min. The MTZ reference tablets released 89.36 % ± 3.64 of their content after 60 min (Fig. 4), while the test products A, B (Fig. 5), and C released 95.79 % ± 3.91, 88.52 % ± 2.52, and 87.79 % ± 5.04 of their contents, respectively, in the same time interval. In addition, the hardness and disintegration times of the stored tablets were determined using the same time intervals. The comparison of the dissolution performance of the different MTZ tablet products was based on the % cumulative medication liberated later 30 min (% Q_{30}) as shown in Fig. 6.

From the data presented in Table 3 and Fig. 6, it can be seen that all the commercial MTZ tablet products stored at 40 °C ± 2 °C/75 % RH ± 5 % for 0.5, 1, 2, and 3 months in their original blister packaging showed a gradual and slight reduction in their mean dissolution efficiency after 30 min (% DE_{30}), as well as a slight increase in their mean dissolution time (MDT), when compared with the same dissolution parameters achieved for the corresponding control tablets.

The similarity (f_2) and difference (f_1) factors calculated for the different MTZ tablet products stored under elevated temperature and humidity conditions are also presented in Table 3.

3.4. *Influence of different packaging forms on the dissolution properties of selected MTZ products stored under accelerated conditions*

The commercial MTZ product (Test C) was selected from the other tablet products to investigate the effect of different packaging forms on the dissolution properties of the tablets when stored for a period of 3 months under stressed conditions. The selection of this product was based on the assumption that the significantly reduced dissolution performance of the Test C tablet product after storage for 3 months under accelerated conditions (Table 3, Fig. 6) would improve if the tablets were additionally packed and inserted into polyethylene bags or child-resistant containers for the purpose of isolation and protection from a high-humidity environment.

The MTZ tablets (Test C) were stored at 40 °C ± 2 °C/75 % RH ± 5 % for 3 months in their original blister packaging (denoted as P1); or in their original blister packaging before being placed inside a polyethylene bag with a zipper (denoted as P2); or in their original blister packaging before being inserted into a child-resistant container (denoted as P3). Differently packed tablets of the Test C product were removed after 3 months of storage and tested for their dissolution performance.

It was noticed that enclosing the original blister packaging of the Test C tablets in a polyethylene bag or in a child-resistant container slightly improved the dissolution profiles of these tablets after 3 months of storage under accelerated conditions when compared with the tablets stored in their original blister packaging. All the tablets stored in the different packaging forms met the USP dissolution requirement by releasing more than 85 % of their content in less than 30 min. The tablets

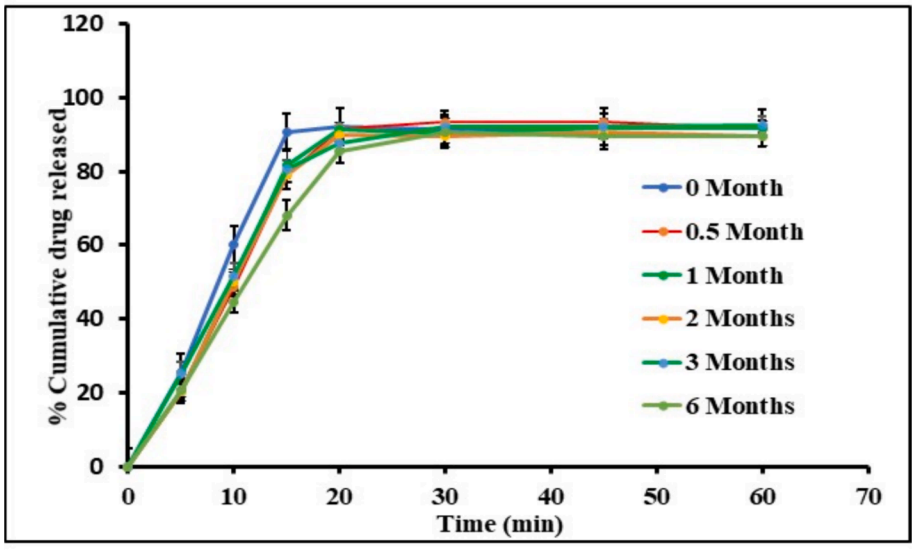

Fig. 4. In vitro dissolution profiles of the reference MTZ tablets (mean ± SD, n = 6) obtained in 0.1 moL L^{-1} HCl from control and after storage at 40 °C ± 2 °C/75 % RH ± 5 % for 6 months.

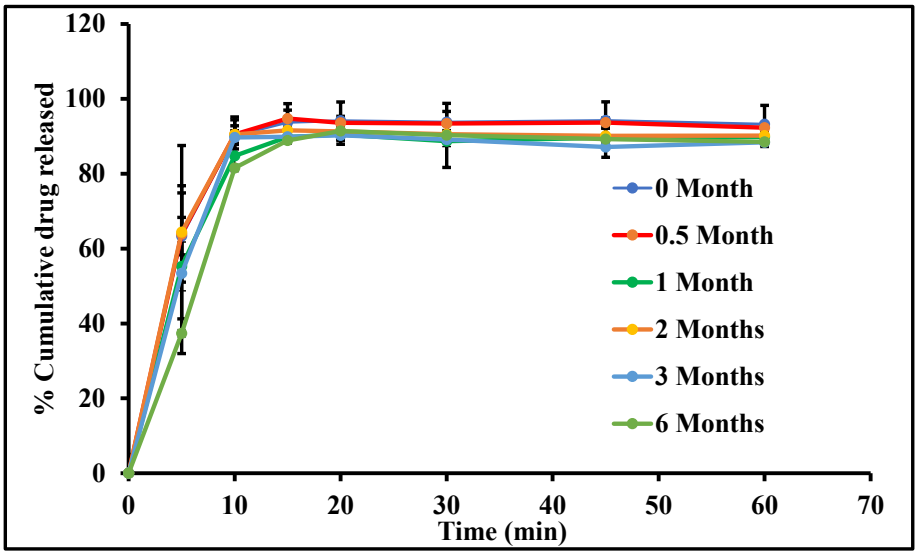

Fig. 5. In vitro dissolution profiles of the test B product (mean ± SD, n = 6) obtained in 0.1. moL L^{-1} HCl from control and after storage at 40°C ± 2 °C/75% RH ± 5% for 6 months.

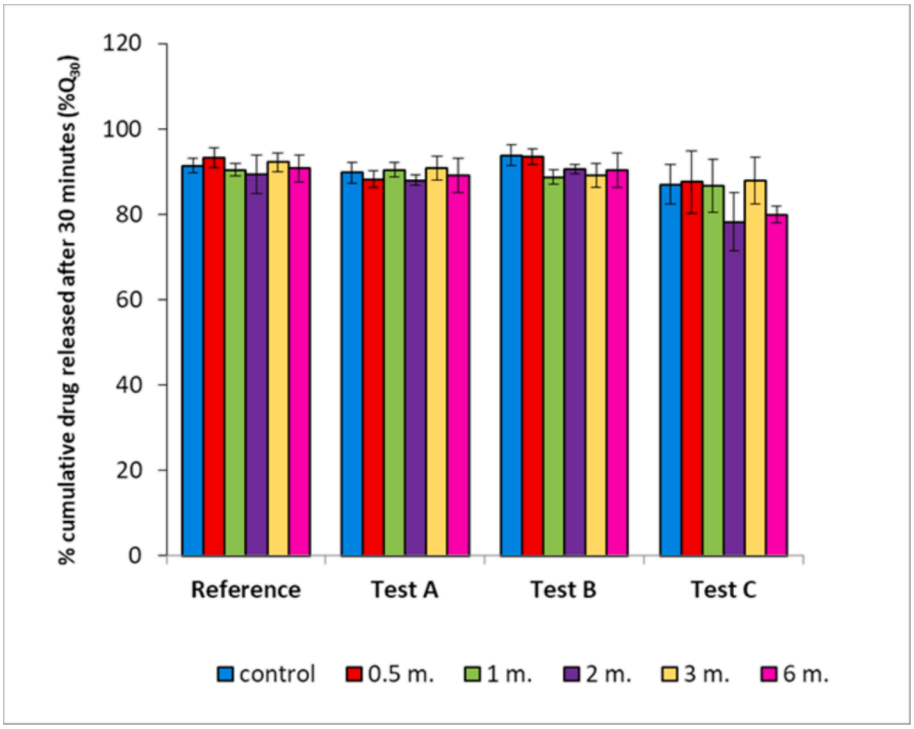

Fig. 6. Comparison of mean cumulative % drug released after 30 min (% Q_{30}) from control and stored MTZ tablets (mean ± SD, n = 6) after storage at 40 °C ± 2 °C/ 75 % RH ± 5 % for 6 months.

stored for 3 months in a polyethylene bag or in a child-resistant container released 90.12 % ± 2.50 and 91.14 % ± 3.91 of their MTZ content after 30 min, respectively, compared to 87.84 % ± 5.45 of the drug released by the tablets stored for 3 months under at the same conditions but without additional packaging. The results of the dissolution parameters obtained for these tablets are presented in Table 4 and Fig. 7.

In order to determine the differences and similarities between the three types of packaging (P1, P2, and P3) used in this part of the study, the fit factors (f_1 and f_2) were calculated, and the obtained results are presented in Table 5.

4. Discussion

4.1. In vitro dissolution of commercial MTZ tablets obtained from different manufacturers

Fig. 3 shows that the MTZ test products A and B demonstrated a faster dissolution rate during the first 10 min of the dissolution interval when compared with the reference product. On the other hand, test product C showed a slower dissolution rate during the first 30 min of the dissolution interval when compared with the reference tablets. This could be due to the type of excipients or inactive ingredients used in tablet formulation in addition to the method of manufacturing (Stuart

Table 3
Hardness, disintegration time, mean dissolution time (MDT), mean dissolution efficiency (%DE_{30}), similarity factor (f_2), and difference factor (f_1) calculated for commercial MTZ tablet products stored at 40 °C ± 2 °C/75 % RH ± 5 % for 6 months, compared to the control tablets.

Product Code	Storage Time (Months)	Hardness (Kg/Cm^2) (Mean ± Sd)	Disintegration Time (Min) (Mean ± Sd)	MDT (Mean ± Sd)	% DE_{30} (Mean ± Sd)	f_2	f_1
Reference	Control	9.59 ± 0.61	7.25 ± 0.83	7.90 ± 1.03	67.63 ± 1.68	––	––
	0.5	16.49 ± 1.41	7.1 ± 0.30	8.96 ± 0.72	63.18 ± 1.57	60.63	5.81
	1	19.67 ± 0.59	7.51 ± 0.79	9.38 ± 1	63.27 ± 0.81	64.11	4.81
	2	23.55 ± 2.20	6.24 ± 0.23	8.91 ± 0.92	62.31 ± 1.02	61.39	6.31
	3	18.48 ± 2.16	8.96 ± 1.39	9.59 ± 1.04	63.49 ± 1.01	64.70	4.62
	6	21.10 ± 1.89*	10.29 ± 0.14*	10.15 ± 0.86*	58.68 ± 2.77*	52.66	10.17
Test A	Control	10.27 ± 0.97	1.31 ± 0.33	1.73 ± 1.03	81.23 ± 1.43	––	––
	0.5	8.88 ± 0.69	1.30 ± 0.01	2.23 ± 0.38	80.39 ± 1.12	91.62	1.10
	1	10.75 ± 1.24	1.11 ± 0.01	2.71 ± 0.86	80.63 ± 1.40	90.33	1.28
	2	10.65 ± 1.01	1.51 ± 0.01	6.82 ± 0.56	79.71 ± 1.39	71.29	3.74
	3	8.90 ± 0.48	1.31 ± 0.01	2.23 ± 0.45	75.33 ± 2.01	81.86	1.70
	6	8.97 ± 1.11	1.13 ± 0.03	6.35 ± 0.75*	72.14 ± 2.91*	73.04	3.13
Test B	Control	12.54 ± 0.52	5.75 ± 0.33	3.95 ± 1.01	80.38 ± 2.85	––	––
	0.5	12.02 ± 1.21	6.30 ± 0.98	4.51 ± 1.03	80.51 ± 1.75	96.33	0.71
	1	12.19 ± 1.36	6.12 ± 0.26	4.84 ± 0.59	75.70 ± 1.34	65.56	5.50
	2	14.02 ± 0.77	5.68 ± 0.54	5.72 ± 0.97	78.99 ± 2.97	78.37	2.69
	3	13.96 ± 1.13	7.03 ± 0.22	4.26 ± 1.84	76.26 ± 2.82	63.44	5.58
	6	14.17 ± 1.09*	8.37 ± 0.05*	5.14 ± 1.32	72.53 ± 3.62*	53.34	8.90
Test C	Control	39.46 ± 0.43	13.26 ± 0.95	13.25 ± 2.71	49.75 ± 4.09	––	––
	0.5	46.6 ± 1.58	12.26 ± 0.14	13.80 ± 2.31	49.01 ± 5.75	90.43	1.55
	1	43.69 ± 3.53	14.49 ± 1.14	16.12 ± 1.65	44.40 ± 2.63	63.35	7.65
	2	47 ± 1.44	13.4 ± 0.01	16.89 ± 2.43	44.04 ± 4.05	62.35	7.16
	3	49.75 ± 1.73	12.96 ± 0.61	16.77 ± 2.16	43.46 ± 4.34	62.53	7.08
	6	50.93 ± 0.32*	15.32 ± 0.36*	15.66 ± 2.38	42.79 ± 1.01*	59.74	9.19

* Significant difference at $p < 0.05$.

Table 4
Mean dissolution time (MDT) and mean dissolution efficiency (% DE30) calculated for Test C MTZ tablets stored at 40oC ± 2 °C /75 % RH ± 5 % for 3 months, in different packaging forms.

Product	Packaging form	MDT (mean ± SD)	% DE_{30} (mean ± SD)
Test C	Control (initial time)	13.25 ± 2.71	49.75 ± 4.09
	P1 [a]	16.77 ± 2.16**	43.46 ± 4.34**
	P2 [b]	12.99 ± 1.23*	53.10 ± 1.84*
	P3 [c]	12.73 ± 1.42*	53.25 ± 1.76*

[a] Original blister packaging.
[b] Original blister packaging inserted in a polyethylene bag with zipper.
[c] Original blister packaging inserted in a child-resistant container.
** $P < 0.05$ (compared with control).
* $P < 0.05$ (compared with P1 [a]).

et al., 2014). This might be reflected in significantly rapid disintegration ($p < 0.05$) of Test A (1.31 ± 0.33 min) and Test B (5.75 ± 0.33 min) tablet products and significantly delayed disintegration ($p < 0.05$) of Test C (13.26 ± 0.95 min) tablet product (Table 2), compared to the disintegration time obtained for the reference tablets (7.25 ± 0.83 min).

The Test C tablet product of MTZ exhibited the lowest dissolution profile (Fig. 3) when compared with the reference and the other MTZ test tablet products (Test A and Test B). This result could be ascribed to the type of excipients used in the tablet formulation, which may be reflected in the hardness of the manufactured tablets. The MTZ tablets (Test C) showed a hardness value of 39.46 ± 0.43 kg/cm^2, which was significantly higher ($p < 0.05$) than the values determined for the reference (9.59 ± 0.61 kg/cm2), Test A (10.27 ± 0.97 kg/cm2), and Test B (12.54 ± 0.52 kg/cm2) tablet products. The high crushing strength values resulted in the significantly delayed disintegration ($p < 0.05$) of the Test C tablet product (13.26 ± 0.95 min) (Table 2) and, therefore, a decreased dissolution profile (Noor et al., 2017).

The obtained values of the fit factors indicate that the MTZ test products (A, B, and C) may not be interchangeable with the reference product. However, it was reported that the calculation of the fit factors may not reveal the variability of different batches because it does not consider uneven spacing between sampling time points and, therefore, may be insensitive to the dissolution profile (Kassaye and Genete, 2013). Therefore, the differences detected by the fit factors calculated for the MTZ test and reference products could be due to the different types of excipients used in the formulation of these tablet products, in addition to the different methods adopted in their manufacturing (Noor et al., 2017). Moreover, the guideline of the US Food and Drug Administration (US FDA) and European Medicines Agency (EMA) (EMA, 2010) on the investigation of bioequivalence stated that "if more than 85 % of the drug is dissolved within 15 min, the dissolution profiles may be accepted as similar" (US-FDA, 2000). The inspection of the dissolution profiles of the MTZ test products (A, B, and C) revealed that these products released 91.65 % ± 2.72 %, 93.72 % ± 1.51 %, and 55.56 % ± 11.08 % of their content, respectively, after 15 min, compared to the 90.75 % ±1.72 % released from the reference tablets in the same time interval. Based on the US FDA and the EMA (EMA, 2010) guidelines, the MTZ test products A and B can be considered similar to the reference tablets. On the other hand, the test product C can be considered different from the reference MTZ tablets, based on the US FDA and EMA guidelines. This difference can be confirmed by the significantly lower value of % DE_{30} and the significantly higher value of MDT ($p < 0.05$) calculated for the MTZ test product C (Table 2) when compared with that observed for the reference tablets. The absence of interchangeability between the generic products and their corresponding reference product has previously been reported for tablets of MTZ (Löbenberg et al., 2012), chloramphenicol (Ferraz et al., 2007), ciprofloxacin (Ngwuluka et al., 2009), and metoprolol tartrate (Polli et al., 1997).

4.2. In vitro dissolution studies of different commercial brands of MTZ stored at high temperatures and relative humidity

It was apparent that all the MTZ tablets stored and tested over a period of 6 months complied with the USP dissolution requirement, which specifies that not less than 85 % of the labeled amount of MTZ must dissolve in 60 min (Fig. 6). Another comparison of the dissolution behavior of the different MTZ tablets stored under the accelerated conditions was carried out based on the dissolution efficiency after 30 min (% DE_{30}) or the mean dissolution time (MDT). These data were calculated and are presented in Table 3. A slight reduction in the

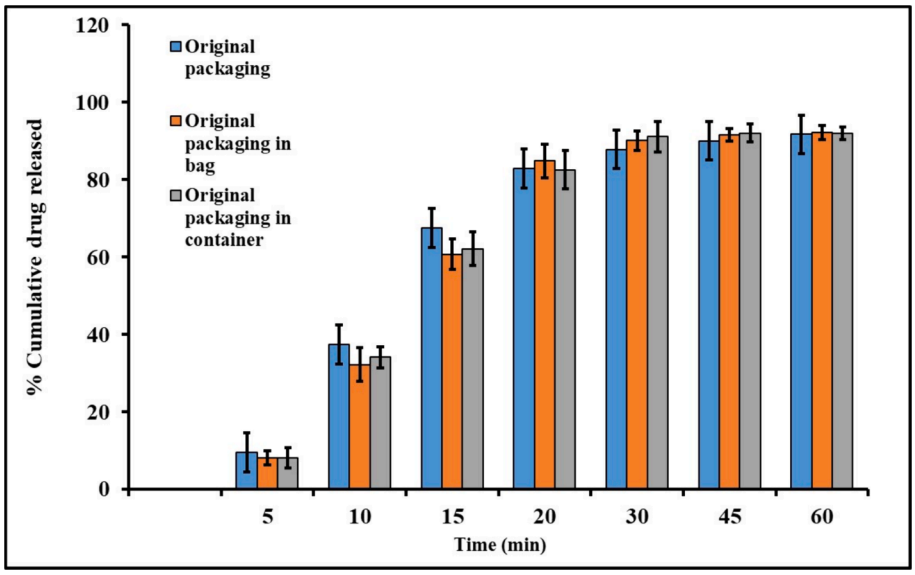

Fig. 7. In vitro dissolution profiles of the test C product (mean ± SD, n = 6) obtained in 0.1. moL L^{-1} HCl stored at 40°C ± 2 °C /75% RH ± 5% for 3 months, in different packaging forms.

Table 5
Difference (f_1) and similarity (f_2) factors calculated for MTZ test product C stored at 40 °C ± 2 °C/75 % RH ± 5 % for 3 months in different packaging forms.

Fit factor	P1 [a] vs. P2 [b]	P1 vs. P3 [c]	P2 vs. P3
f_1	4.22	3.44	1.53
f_2	73.12	77.14	90.28

[a] Original blister packaging.
[b] Original blister packaging inserted in a polyethylene bag with zipper.
[c] Original blister packaging inserted in a child-resistant container.

dissolution performance of these commercial MTZ tablets continued as the time of storage progressed. The slight changes in the dissolution parameters could be explained based on the fact that MTZ is a relatively stable material (Wu and Fassihi, 2005). Good stability and negligible degradation were reported for MTZ when it was stored in its solid state under accelerated conditions (40 °C ± 2 °C/75 % RH ± 5 %) for a period of 3 months (Wu and Fassihi, 2005).

Also, the data presented in Table 3 show that the reduction in the mean dissolution efficiency after 30 min (% DE_{30}) was more prominent for all the MTZ tablet products after storage for a period of 6 months under accelerated conditions. For example, the values of the % DE_{30} calculated for the control tablets of the reference (67.63 % ± 1.68), Test A (81.23 % ± 1.43), Test B (80.38 % ± 2.85), and Test C (49.75 % ± 4.09) decreased significantly ($p < 0.05$) to 58.68 % ± 2.77, 72.14 ± 2.91, 72.53 % ± 3.62, and 42.79 % ± 1.01, respectively. The use of different excipient combinations in the manufacture of the various tablets could be attributed to this observation. These excipients might have interacted differently with the active ingredient during storage under stressed conditions, leading to reduced dissolution properties (Stuart et al., 2014).

Inspection of the leaflets included with commercial MTZ tablet products used in this study (reference, Test A, and Test B) revealed different types of excipients in each product (Table 1). The presence of different excipients (like magnesium stearate, povidone, or specific coating material) in different products may have contributed to reduced %DE_{30} of MTZ tablets after storage for 6 months under stressed conditions. On the other hand, the leaflet of Test C product did not specify the excipients used in this tablet product. A request made to the manufacturing company of this product to provide the list of excipients used was not approved.

It was reported that some excipients like sodium lauryl sulfate, sorbitol and propylene glycol may exert a potential effect on the bioavailability of certain MTZ formulations (Rediguier et al., 2011). Also, the presence of magnesium stearate in some MTZ products may affect their dissolution profiles (Stuart et al., 2014). The addition of povidone as a binder in tablet formulation may result in a slower dissolution rate and hence a reduced dissolution efficiency (Block et al., 2008). In addition, the type of the coating material may affect the dissolution behavior of coated tablets. For example, certain types of the coating material may contain pH sensitive substances which may cause poor dissolution at pH 1.2 (Stuart et al., 2014).

It is also possible that some soluble excipients used in tablet formulation might have absorbed moisture during storage at high humidity conditions (Chowhan, 1979). Moisture may transfer through packaging materials like polyvinyl chloride blister packaging and affect tablet crushing strength depending on other formulation components (Akala, 2008). It was reported that after removal of tablets from the high humidity environment and storage at ambient room temperature, partial loss of the absorbed moisture (by recrystallization) may take place leading to increased hardness and increased disintegration time of tablets (Chowhan, 1979). Therefore, package moisture permeability should be considered together with formulation excipients when selecting a package for a given product (Akala, 2008).

Based on these facts, the reduced values of % DE_{30} obtained for the different commercial MTZ tablets after storage for 6 months under accelerated conditions (Table 3) could be attributed to the increased hardness and disintegration time of the stored tablets, which resulted in decreased drug dissolution. The inspection of the results of the hardness and disintegration time presented in Table 3 revealed a gradual increase in the hardness values and the disintegration time values of the tablets stored over a period of 3 months when compared with the values obtained for the control tablets. Increased hardness and disintegration time appeared more clearly after 6 months of storage for all the commercial tablets. For example, the hardness value determined for the Test C control tablets increased significantly ($p < 0.05$) from 39.46 ± 0.43 Kg/Cm^2 to 50.93 ± 0.32 Kg/Cm^2, while the disintegration time increased significantly ($p < 0.05$) from 13.26 ± 0.95 min to 15.32 ± 0.36 min after 6 months of storage. These observations were generally noticed for the reference and for the test products B and C, although they were sometimes inconsistent. In the case of the Test A tablet product, slight changes in the disintegration time and hardness were observed

throughout the whole period of storage, and this could be due to the different types of excipients used in this product.

It can be seen that the values of the similarity factor (f_2) calculated for all the tablet products stored for 6 months under accelerated conditions were more than 50, indicating the similarity of the dissolution profiles of the stored tablets to their corresponding control tablets. These similarities were confirmed by the difference factor (f_1) calculated for all the stored tablets; this was found to be less than 15. In addition, a slight reduction in the mean % DE_{30} and a slight increase in the MDT of the stored MTZ tablets compared to the same dissolution parameters calculated for their corresponding control tablets (Table 3) may further confirm the similarity of the stored and control MTZ tablets as well as the relatively good stability of the active ingredient, MTZ.

In addition, the Test C tablet product of MTZ exhibited the lowest dissolution efficiency after 30 min (% DE_{30}) and the highest mean dissolution time (MDT) after storage for 3 months under accelerated conditions (Table 3), when compared with the other commercial MTZ products stored for the identical period in an unchanged environment. This could be due to the different excipients used in the formulation of this tablet product, which might have interacted with the active ingredient during storage and caused reduced dissolution properties (Stuart et al., 2014).

4.3. Influence of different packaging forms on the dissolution properties of selected MTZ products stored under accelerated conditions

It is obvious that the additional packaging of MTZ tablets (Test C) in polyethylene bags or in child-resistant containers resulted in a significant increase ($p < 0.05$) in the values of %DE_{30} (53.10 % ± 1.84 and 53.25 % ± 1.76) calculated for the two packaging forms, respectively, when compared with the value calculated for the tablets stored without additional packaging (43.46 % ± 4.34) (Table 4). Moreover, the use of polyethylene bags or child-resistant containers resulted in a significant ($p < 0.05$) decrease in the MDT values of the tablets stored in their original packaging, from 16.77 ± 2.16 min to 12.99 ± 1.23 min and 12.73 ± 1.42 min, respectively (Table 4). These observations indicate an improvement in the dissolution properties of the tablets with additional packaging.

The comparison of the dissolution profiles of the Test C tablets stored for 3 months in their original packaging with those of the tablets stored in additional polyethylene bags or child-resistant containers revealed difference factors (f_1) of 4.22 and 3.44, respectively (Table 5). These values of (f_1) are less than 15 and therefore indicate that the protective effects exerted by the additional packaging of the tablets in polyethylene bags (P1 vs. P2) or child-resistant containers (P1 vs. P3) are similar to those exerted by the original blister packaging, despite the significant increase in the values of % DE_{30} obtained by using the two packaging forms (Table 4). Also, comparing the dissolution profiles obtained for the MTZ tablets stored in additional polyethylene bags to those obtained for the tablets stored in child-resistant containers (P2 vs. P3) revealed a difference factor (f_1) of 1.53 (Table 5). This value means that there was no difference in the protective effect exerted by either polyethylene bags or child-resistant containers.

The inspection of the similarity factor (f_2) presented in Table 5 revealed similar observations to those explained above, which were based on the difference factor (f_1). The calculated value of f_2 (90.28) proved that the protective effect exerted by polyethylene bags is similar to that produced by child-resistant containers (P2 vs. P3).

Generally, different packaging materials (e.g., polyethylene bags, paper bags, aluminum bags, glass, or plastic containers) may affect the dissolution properties of the solid dosage forms stored under elevated temperature and humidity conditions in different ways. This is because certain factors like the type of the active ingredient, the excipients, and the storage conditions may contribute to the determination of the packaging suitability and its effects on the properties of a given dosage form (Akala, 2008). For example, the dissolution properties of different brands of film-coated erythromycin stearate tablets stored in different packaging materials were investigated under different storage conditions (50 °C, 50 % RH and 40 °C, 90 % RH) (Gouda et al., 1980). All the stored tested tablets showed significantly reduced dissolution rates under both storage conditions. However, the tablets stored in glass containers exhibited higher dissolution rates compared to the tablets stored in paper or plastic containers. These findings indicated that glass containers have a better protection effect relative to other types of packaging materials (Gouda et al., 1980). Also, the press-through packaging sheets of indomethacin capsules stored under accelerated conditions for 45 days in either polyethylene bags or aluminum bags with zippers showed average 20- minute dissolution rates of 62.7 % and 98.6 %, respectively (Kuribayashi et al., 2018). The reduced dissolution rate of the capsule sheet inserted in polyethylene bags was attributed to the passage of water molecules from the storage environment through the polyethylene bags, while the enhanced dissolution rate of the tested capsules approached that obtained for the capsule sheets stored at room temperature. The authors concluded that the aluminum bags were more protective for capsule sheets than the polyethylene bags (Kuribayashi et al., 2018).

5. Conclusion

This study evaluated the effects of accelerated storage conditions on the dissolution characteristics of commercially available MTZ tablets, including a reference product and three generic or test products coded as A, B, and C. All the MTZ control tablets met the USP dissolution requirement as not less than 85 % of the labeled amount of MTZ was dissolved in 60 min. The MTZ reference tablets released 91.79 % ± 1.23 of their content after 60 min, while the test products A, B, and C released 87.96 % ± 2.60, 93.26 % ± 2.01, and 88.61 % ± 2.04 of their contents, respectively, in the same time interval. The MTZ test products A and B were considered similar to the reference product, while test product C was found to be different from the reference product. The MTZ tablet products stored for 6 months at 40 °C ± 2 °C/75 % RH ± 5 % maintained compliance with the USP dissolution requirement for MTZ tablets but with slight changes in the dissolution parameters that became more prominent as the storage time progressed. The use of additional packaging materials resulted in the improvement of the dissolution parameters of the stored tablets. Therefore, it should be emphasized that storage conditions which are good enough to keep the active ingredient's medicinal properties intact during the shelf life of medicines must be ensured. The exposure of pharmaceutical products to different temperature and humidity conditions during their long journey from the manufacturer to the consumer requires strict adherence to the labeled storage conditions to ensure the protection of the product in areas that require special storage conditions, such as hot and/or humid areas. In addition, the study sheds the light to the effect of storage conditions such as humidity and temperature on the in vitro dissolution performance of MTZ marketed tablet products, which could affect the efficacy and patients' safety of the loaded APIs.

CRediT authorship contribution statement

Basmah N. Aldosari: Conceptualization, Data curation, Writing – original draft, Writing – review & editing, Visualization, Investigation, Formal analysis, Methodology, Project administration, Software. **Areej M. Al-Mutairi:** Writing – original draft, Investigation, Validation, Formal analysis, Methodology, Resources. **Alanood S. Almurshedi:** Investigation, Review & editing, Data curation, Resources. **Iman M. Alfagih:** Visualization, review & editing, Formal analysis. **Bushra T. Al Quadeib:** Review & editing, Formal analysis. **Eram Eltahir:** Methodology. **Salma S. Almarshidy:** Methodology. **Mohamed A. Ibrahim:** Investigation, Visualization, Writing – review & editing. **Amal El Sayeh F. Abou El Ela:** Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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