*FABAD J. Pharm. Sci., 33, 87–94, 2008*

*RESEARCH ARTICLE*

# Comparison of Dissolution Profiles of Two Commercially Available Co-Trimoxazole Tablets

Esin SAYAR*,**, Selma ŞAHİN*°, Şemsettin CEVHEROĞLU**, A. Atilla HINCAL*

***Comparison of Dissolution Profiles of Two Commercially Available Co-Trimoxazole Tablets***

***Summary***

*The dissolution test is intended to measure the time required for a given drug in an oral solid dosage form to go into solution under specified set of conditions. It is used to assess the batch-to-batch quality of a drug product, guide to development of a new formulation, ensure continuing product quality, support the bioavailability of a new product, bioequivalence of an essentially similar product. The aim of this study was to investigate the influence of dissolution medium on the in vitro release of trimethoprim/sulfamethoxazole (TMP/SMX: 160/800 mg) from, two commercially available Co-trimoxazole tablets. Three different buffers (pH 1.2, 4.6 and 6.8) were used as the dissolution medium (900 mL) and the paddle rotation speed was kept at 50 rpm. The dissolution profiles of both tablets were compared using difference ($f_1$) and similarity ($f_2$) factors. Release of both TMP and SMX from Co-trimoxazole tablets was found to be pH dependent. Although the dissolution profiles of TMP obtained for test and reference products at pH 1.2 medium (50 rpm) was similar, based on $f_1$ and $f_2$ values, the dissolution profiles of TMP were dissimilar for other conditions (pH 4.6 and 6.8 with 50 rpm). On the other hand, for all three dissolution media given above, all dissolution profiles of SMX obtained for test and reference formulations were different ($f_1 > 25$ and $f_2 < 37$ for all mediums). All these results indicate that dissolution studies are not sufficient to demonstrate the similarity of these tablet formulations, and that further in vivo studies are required.*

***Key Words:*** *Dissolution, Trimethoprim, Sulfamethoxazole, Co-trimozaxole*

*Received: 26.11.2009*
*Revised: 05.02.2010*
*Accepted: 12.02.2010*

***Piyasada Mevcut İki Kotrimoksazol Tabletin Dissolüsyon Profillerinin Karşılaştırılması***

***Özet***

*Çözünme testi bir ilacın belirlenmiş koşullar altında bir oral katı dozaj şeklinden çözeltiye geçmesi için gerekli olan sürenin ölçülmesini amaçlamaktadır. Bir ilaç ürününün seriden seriye kalitesini ölçmek, yeni bir formülasyonun geliştilmesine rehberlik etmek, ürün kalitesinin devamını sağlamak, yeni bir ürünün biyoyararlanımını ve esasta benzer bir ürünün biyoeşdeğerliğini desteklemek amacıyla kullanılır. Bu çalışmanın amacı, piyasada mevcut olan iki kotrimoksazol tabletten trimetoprim/sülfametoksazol (TMP/SMX: 160/800 mg)'ün in vitro salımı üzerine çözünme ortamlarının etkisini incelemektir. Çözünme ortamı (900 mL) olarak üç farklı tampon (pH 1.2, 4.6 and 6.8) kullanılmış ve pedal dönme hızı 50 rpm'ye ayarlanmıştır. Her iki tabletin çözünürlük profilleri fark ($f_1$) ve benzerlik ($f_2$) faktörleri kullanılarak karşılaştırılmıştır. TMP ve SMX"in kotrimoksazol tabletlerinden salımının pH'ya bağımlı olduğu bulunmuştur. pH 1.2 ortamında (50 rpm) test ve referans ürünlerden TMP'nin çözünürlük profilleri benzer olmasına rağmen, diğer koşullarda (pH 4.6 and 6.8) TMP'nin çözünürlük profilleri benzer değildir. Diğer taraftan, yukarıda verilen üç çözünürlük ortamında test ve referans formülasyonlarından SMX için elde edilen dissolüsyon profilleri farklıdır ( tüm ortamlar için $f_1 > 25$ ve $f_2 < 37$). Tüm bu sonuçlar bu tablet formülasyonlarının benzerliğini göstermek için çözünürlük profillerinin yeterli olmadığını ve ilave in vivo çalışmaların gerektiğini göstermektedir.*

***Anahtar Kelimeler:*** *Dissolüsyon, Trimetoprim, Sülfametoksazol, Kotrimoksazol*

* Hacettepe University, Faculty of Pharmacy, Ankara, Turkey
** Ministry of Defense, Army Drug Factory, Ankara, Turkey
° Corresponding author E-mail: selmas@hacettepe.edu.tr

## INTRODUCTION

Drug absorption from a solid dosage form after oral administration depends on the release of drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance. Based on this general consideration, in vitro dissolution test for immediate release solid oral dosage forms (i.e. tablets, capsules) is used to assess the batch-to-batch quality control of a drug product; guide the development of new formulations; and ensure the sustainability of the product quality and performance after certain changes, such as those in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process. Dissolution test can also be used to support the bioavailability of a new product, the bioequivalence of an essentially similar product or variations (1,2).

The biopharmaceutics classification system (BCS) is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate release dosage forms: dissolution, solubility and intestinal permeability. According to the BCS, the drug substances are classified as class 1 (high solubility and high permeability), 2 (low solubility and high permeability), 3 (high solubility and low permeability) and 4 (low solubility and low permeability). In addition, immediate release (IR) oral dosage forms are categorized as having rapid or slow dissolution. When the in vivo dissolution of an IR solid oral dosage form is rapid in relation to the gastric emptying and the drug has high permeability, the rate and extent of drug absorption is unlikely to be dependent on dissolution and/or gastrointestinal transit time. Under such circumstances, demonstration of in vivo bioavailability or bioequivalence may not be necessary for highly soluble and highly permeable class 1 substances in IR solid oral dosage form that exhibit rapid in vitro dissolution using the recommended test methods (3).

The combination of trimethoprim (TMP) and sulfamethoxazole (SMX) in a 1:5 ratio, known as Co-trimoxazole, has been used for the treatment of a wide variety of infections due to Gram-positive and Gram-negative organisms; particularly those of the urinary, respiratory, and gastro-intestinal tracts (4-6). The synergistic bactericidal effect of the combination is due to sequential blockade of bacterial enzyme systems associated with tetrahydrofolate synthesis. SMX inhibits bacterial synthesis of dihydrofolic acid by competing with paraaminobenzoic acid. TMP blocks the production of tetrahyrofolic acid from dihydrofolic acid by binding to and reversibly inhibiting the dihydrofolate reductase (7,8). Based on BCS (9) and biopharmaceutics drug disposition classification system (10), SMX is a class 2 compound, whereas, TMP is a class 3 compound.

Co-trimoxazole was originally formulated as tablets in two strengths (80/400 mg and 160/800 mg TMP/SMX). Furthermore, several generic formulations are commercially available in the Turkish Drug Market. The objective of this study was to investigate the influence of dissolution medium on the in vitro release of TMP/SMX (160/800 mg) from two commercially available Co-trimoxazole tablets.

## MATERIALS AND METHODS

### Materials

TMP was a BP reference standard and SMX was a USP reference standard. Acetonitrile and methanol were of HPLC grade and purchased from Merck. All other chemicals were of analytical grade and obtained from Merck.

### Quality Control Tests for the Test and Reference Tablets

Dissolution studies on the test (Batch no:0412522001) and reference (Bactrim® 160/800mg, TMP/SMX; Batch no:B1105) tablets were conducted using USP Apparatus II (paddle method) with twelve replicates at 37 ± 0.5°C (Sotax, Switzerland). Three different buffers (pH 1.2, 4.6 and 6.8) were used as the dissolution medium (900 mL) and the paddle

rotation speed was kept at 50 rpm. 0.1N HCl was used as the pH 1.2 buffer. The pH 4.6 buffer consisted of 0.2M potassium biphthalate (50 mL), 0.2M sodium hydroxide (11.1 mL) and distilled water (q.s. 200 mL). On the other hand, the pH 6.8 buffer consisted of 0.2M potassium phthalate monobasic (50 mL), 0.2M sodium hydroxide (22.4 mL) and distilled water (q.s. 200 mL) (11). Additionally, in accordance with the USP method, dissolution studies with test and reference tablets were performed in 900 mL of pH 1.2 buffer at a paddle stirring rate of 75 rpm (11). In all experiments, at predetermined time intervals (5, 10, 15, 30, 45 and 60 min), 2 mL sample was withdrawn and replaced with an equal volume of fresh medium to maintain a constant total volume. After the filtration and dilution of the dissolution samples with the mobile phase, the concentrations of TMP and SMX were determined simultaneously by a validated HPLC method.

In addition, weight variation, diameters and thickness, hardness, friability, disintegration time, and content uniformity of active ingredients (TMP and SMX) were determined for the quality control of test and reference tablets. For the determination of weight variation, 20 tablets were weighed individually on an analytical balance (Sartorious, Germany), and then mean and standard deviation were calculated for test and reference tablets. Diameters and thickness of the tablets were determined from 20 tablets with an electronic compass (Bochem, Germany). Hardness of 10 tablets were measured using a hardness tester (Pharma Test, Germany). Dusted and accurately weighed 20 tablets were placed in a friabilator (Pharma Test, Germany) and rotated 4 min (100 revolution). To remove adhering particles, the tablets were dusted again and then weighed. The mean percent friability was calculated from the difference in tablet weights. The disintegration time of tablets (n=6) was determined at 37°C in water using disintegration tester (Komet, Turkey). Content uniformity of test and reference tablets was performed as described in the official monograph. Briefly, twenty tablets were weighed and finely powdered. TMP and SMX amounts were then determined from the accurately weighed portion of the powder, equivalent to about 160 mg of SMX (11).

**HPLC Analysis of Trimethoprim/ Sulfamethoxazole**

The HPLC system operating in isocratic mode, consisted of Waters (USA) equipments including a 515 solvent delivery system, a Waters 717 plus autosampler, and a Waters 996 Photodiode array detector. Reverse phase $C_8$ column packed with 5 µm dimethyl octadecylsilyl bonded amorphous silica (4.6 mm × 250 mm) was used as the stationary phase. Chromatographic analysis was carried out at ambient temperature. The compounds were separated using a mobile phase consisted of acetonitrile:water:acetic acid (50:49:1). The mobile phase was prepared daily, filtered (0.45 µm), sonicated before use, and delivered at a flow rate of 1 mL/min. The detector responses were set at 254 nm. The amount of the compounds dissolved were determined using calibration curves constructed over the range of 8 to 25 µg/mL for TMP and 40 to 120 µg/mL for SMX. The proposed method was validated as to selectivity, sensitivity, linearity and precision (system repeatability and intra-day repeatability).

**Data Analysis**

Dissolution profiles were compared using two model-independent parameters: the difference ($f_1$) and similarity ($f_2$) factors (1). The difference factor ($f_1$) calculates the percent (%) difference between the two curves at each time point and is a measurement of the relative error between the two curves (1):

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} x100$$

where $n$ is the number of time points, $R_t$ is the dissolution value of the reference batch at time t, and $T_t$ is the dissolution value of the test batch at time t.

The similarity factor ($f_2$) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves (1).

$$f_2 = 50x\log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n}\left(R_t - T_t\right)\right]^{-0.5} x\ 100\right\}$$

## RESULTS AND DISCUSSIONS

### Validation of the Analytical Method

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice (12). In this study, the HPLC method used for simultaneous determination of TMP and SMX was validated with respect to selectivity, sensitivity, linearity and precision. TMP and SMX were well separated with retention times of 2.7 min for TMP and 4.3 min for SMX, with no interfering peaks in the chromatograms. The calibration curves were linear for both TMP (8-25 µg/mL) and SMX (40-120 µg/mL) with determination coefficients always greater than 0.999. The relative standard deviations calculated for the precision of the system repeatability were lower than 2% for both TMP and SMX. The relative standard deviations and Bias values estimated for the intra-day repeatability of the assay were within the acceptable limits (< 2%) indicating that the precision and accuracy of the proposed HPLC method were satisfactory. The detection and quantification limits were 0.38 and 1.24 µg/mL for SMX, 0.55 and 1.53 µg/mL for TMP, respectively. All the results clearly indicate that the developed HPLC method was precise, accurate, specific and sensitive enough for simultaneous determination of TMP and SMX in dissolution samples.

### Quality Control Tests

The quantitative evaluation and assessment of a tablet's chemical, physical and bioavailability properties are important in the design of tablets and to monitor product quality. These properties are important since chemical breakdown or interactions between tablet components may alter the physical tablet properties, and greatly affect the bioavailability of the tablet system. There are various standards that have been set regarding the quality of pharmaceutical tablets. These include the diameter, size, shape, thickness, weight, hardness, disintegration and dissolution characters. The diameters and shape depends on the die and punches selected for the compression of tablets. The remaining specifications assure that tablets do not vary from one production lot to another (13). The quality control values determined for the test and reference tablets are summarized in Table 1. Although friability and disintegration of the test formulation were slightly higher than those of the reference product, all quality control parameters were within the specified limits (e.g. weight variation <5%, friability <1% and disintegration time <15 min).

For poorly soluble drugs, the rate limiting step in the absorption process is the dissolution rate. Therefore, determination of dissolution rate can be a useful guide for comparative bioavailability.

**Table 1.** Quality control values (mean ± SD) obtained for the test and reference tablets.

| Parameter | Test | Reference |
|---|---|---|
| **Weight (mg)*** | 1166.0 ± 19.9 | 1024.2 ± 18.9 |
| **Hardness (N)**** | 153±9.3 | 158±5.8 |
| **Friability (%)** | 0.36 | 0.004 |
| **Width (mm)*** | 8.84 ± 0.01 | 8.92 ± 0.02 |
| **Length (mm)*** | 19.1 ± 0.01 | 19.1 ± 0.02 |
| **Thickness (mm)*** | 8.05 ± 0.02 | 7.29 ± 0.03 |
| **Disintegration time (min)***** | 2.83±0.35 | 1.42±0.3 |
| **Content of active ingredient (%)** | TMP: 101.3<br>SMX: 99.1 | TMP: 99.3<br>SMX: 99.8 |

*n=20; **n=10; ***n=6

Since drug absorption and physiological availability depend on the availability of the drug substance in the dissolution state, having suitable dissolution characteristics is important for a satisfactory tablet. The dissolution test measures the amount of time required for certain percentage of the drug substance in a tablet to go into solution under a specified set of conditions. It describes a step towards physiological availability of the drug substance, but it is not designed to measure the safety or efficacy of the tablet being tested. It provides in vitro control procedure to eliminate variation among production batches. The dissolution medium must be aqueous and the pH of the medium should be controlled and should simulate the biological conditions (14,15).

The results of the dissolution studies were summarized in Table 2 for TMP and Table 3 for SMX. Corresponding dissolution profiles were given in Figures 1 and 2, respectively. It can be seen from the results that dissolution of both TMP and SMX from Co-trimoxazole tablets was pH dependent. With an increase in the pH of the dissolution media from 1.2 to 6.8, release of TMP from test and reference products was decreased. In the case of SMX, dissolved amount was decreased initially when the pH of the dissolution medium rose from 1.2 to 4.6, probably due to a decrease in the surface ionization at this pH, and then increased with and increase of the dissolution medium to 6.8. These observations are in accordance with the solubilities of TMP and SMX. Although solubility of TMP decreases as a function of pH (from 154.1 to 55.1 mg/mL for pH 1.2 to 6.8, respectively), SMX shows similar pattern in solubility too (506 mg/mL for pH 1.2; 159 mg/mL for pH 4.6 and 670 mg/mL for pH 6.8) (16).

The dissolution profiles of test and reference products

**Table 2.** Percent dissolved for TMP as a function of time.

| Time (min) | Percent Dissolved | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Test | | | | Reference | | | |
| | pH 1.2 (USP)* | pH 1.2** | pH 4.6** | pH 6.8** | pH 1.2 (USP)* | pH 1.2** | pH 4.6** | pH 6.8** |
| 5 | 92.8 ± 4.7 | 79.4 ± 11.0 | 42.5 ± 7.1 | 16.9 ± 3.1 | 68.5 ± 23.7 | 73.6 ± 8.3 | 26.1 ± 5.0 | 10.6 ± 2.2 |
| 10 | 99.1 ± 3.1 | 77.2 ± 7.0 | 56.7 ± 8.4 | 28.6 ± 3.0 | 91.1 ± 2.2 | 78.2 ± 6.9 | 37.3 ± 9.0 | 17.9 ± 2.6 |
| 15 | 98.6 ± 1.3 | 83.3 ± 4.4 | 67.1 ± 5.1 | 37.9 ± 2.5 | 90.7 ± 2.6 | 83.1 ± 4.6 | 48.3 ± 7.4 | 25.4 ± 2.5 |
| 30 | 98.7 ± 9.1 | 84.6 ± 4.8 | 74.5 ± 8.4 | 57.0 ± 4.7 | 92.3 ± 1.4 | 89.8 ± 3.3 | 63.2 ± 10.0 | 41.8 ± 9.3 |
| 45 | 95.5 ± 3.1 | 86.4 ± 3.2 | 80.6 ± 4.9 | 68.3 ± 4.4 | 88.2 ± 6.8 | 91.1 ± 2.5 | 70.7 ± 9.7 | 65.1 ± 7.2 |
| 60 | 95.1 ± 2.9 | 87.2 ± 2.8 | 82.4 ± 4.9 | 73.1 ± 7.0 | 91.3 ± 4.5 | 89.5 ± 5.4 | 78.3 ± 7.5 | 70.5 ± 5.8 |

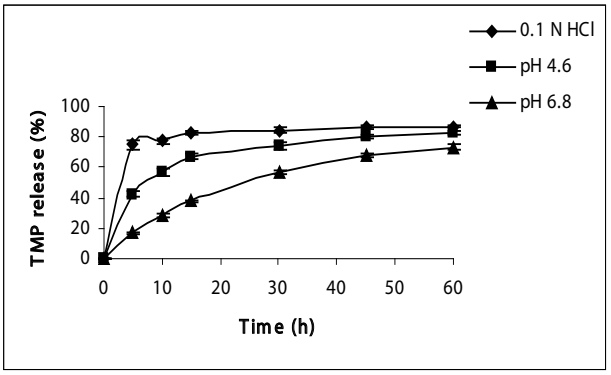
* 75 rpm (mean ± SD; n=6); **50 rpm (mean ± SD; n=12)

**Table 3.** Percent dissolved for SMX as a function of time.

| Time (min) | Percent Dissolved | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Test | | | | Reference | | | |
| | pH 1.2 (USP)* | pH 1.2** | pH 4.6** | pH 6.8** | pH 1.2 (USP)* | pH 1.2** | pH 4.6** | pH 6.8** |
| 5 | 78.3 ± 5.0 | 61.3 ± 10.3 | 32.7 ± 5.8 | 44.5 ± 6.4 | 41.7 ± 15.7 | 23.8 ± 3.7 | 9.6 ± 2.0 | 18.2 ± 3.5 |
| 10 | 91.1 ± 1.9 | 68.5 ± 6.6 | 41.1 ± 7.1 | 55.9 ± 5.4 | 80.6 ± 5.4 | 36.4 ± 2.9 | 14.4 ± 3.7 | 29.4 ± 3.6 |
| 15 | 93.5 ± 0.6 | 77.4 ± 4.2 | 49.2 ± 3.3 | 62.1 ± 4.5 | 92.0 ± 4.3 | 49.0 ± 2.1 | 21.3 ± 3.5 | 39.5 ± 3.0 |
| 30 | 94.1 ± 3.1 | 82.6 ± 4.5 | 56.3 ± 8.4 | 73.9 ± 5.9 | 100.0 ± 2.5 | 71.2 ± 5.3 | 33.8 ± 3.9 | 53.7 ± 11.4 |
| 45 | 96.9 ± 3.9 | 85.1 ± 3.5 | 61.5 ± 3.9 | 80.3 ± 5.6 | 96.9 ± 7.7 | 79.6 ± 5.3 | 41.2 ± 2.8 | 75.0 ± 7.3 |
| 60 | 95.7 ± 2.4 | 87.0 ± 4.6 | 61.9 ± 6.3 | 81.5 ± 7.7 | 100.1 ± 4.6 | 83.0 ± 8.0 | 50.1 ± 2.5 | 77.9 ± 6.6 |

* 75 rpm (mean ± SD; n=6); **50 rpm (mean ± SD; n=12)

A.




B.

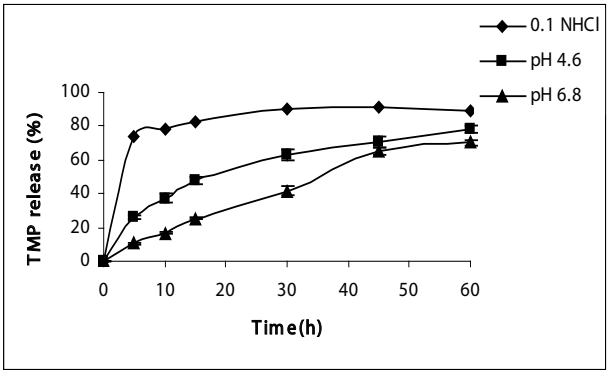
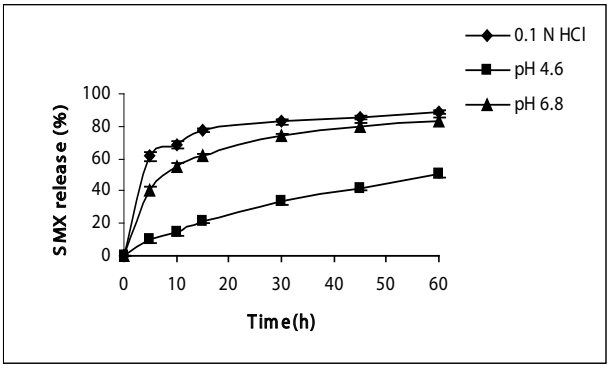



**Figure 1.** Mean (± SD) in vitro dissolution profiles of TMP from test (A) and reference (B) products. Three different buffers (pH 1.2, 4.6 and 6.8) were used as the dissolution medium (900 mL) and the paddle rotation speed was kept at 50 rpm.

**A.**




**B.**

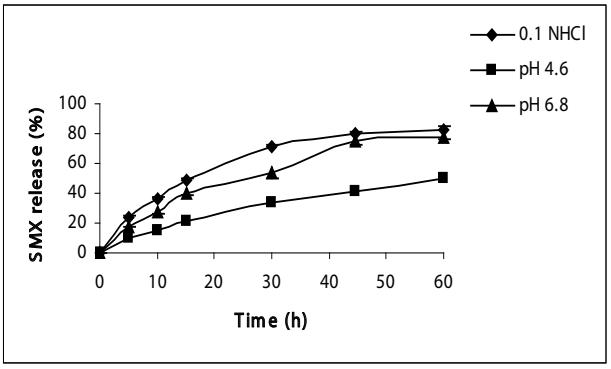



**Figure 2.** Mean (± SD) in vitro dissolution profiles of SMX from test (A) and reference (B) products. Three different buffers (pH 1.2, 4.6 and 6.8) were used as the dissolution medium (900 mL) and the paddle rotation speed was kept at 50 rpm.

were compared using difference ($f_1$) and similarity ($f_2$) factors. When calculated values of $f_1$ lie between 0 and 15 and those of $f_2$ lie above 50, the dissolution profiles are interpreted to be similar (1,3). When the USP method (pH 1.2, 75 rpm) was used, both TMP and SMX in test and reference products dissolved more than 85% of the labeled amount of the drug in 15 min, the profile comparison with and $f_1$ and $f_2$ tests was, thus, unnecessary (Tables 2 and 3). Although the dissolution profiles of TMP obtained for test and reference products at pH 1.2 medium (50 rpm) were similar, based on $f_1$ and $f_2$ values, the dissolution profiles of TMP were dissimilar for other conditions (pH 4.6 and 6.8 with 50 rpm; Table 4). In the case of SMX, for all three dissolution media given above, all dissolution profiles obtained for test and reference formulations were different ($f_1 > 25$ and $f_2 < 37$ for all mediums; Table 4). All these results indicate that dissolution studies are not sufficient to demonstrate the similarities of these tablet formulations, and that further in vivo studies are required.

## CONCLUSIONS

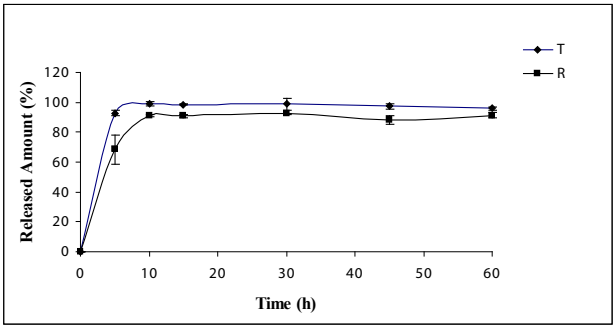
The developed HPLC method was precise, accurate, specific and sensitive enough for simultaneous determination of TMP and SMX from dissolution samples. In FDA guidance (3), an immediate release

**Table 4.** Difference ($f_1$) and similarity ($f_2$) values obtained for TPM and SMX.

| Medium (50 rpm) | TMP | | SMX | |
|---|---|---|---|---|
| | $f_1$ | $f_2$ | $f_1$ | $f_2$ |
| **pH 1.2** | 3.1 | 72.8 | 25.5 | 34.9 |
| **pH 4.6** | 21.4 | 42.7 | 68.0 | 32.3 |
| **pH 6.8** | 20.0 | 49.5 | 28.4 | 36.9 |

drug product is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 30 min, using U.S. Pharmacopeia Apparatus II at 50 rpm (or Apparatus I at 100 rpm) in a volume of 900 mL or less in each of 0.1N HCl, pH 4.6 and pH 6.8 media. Therefore, all dissolution studies except the USP method (75 rpm), were performed in three different buffers (pH 1.2, 4.6, 6.8) at 50 rpm. Dissolution profiles of both TMP and SMX were pH-dependent. When the USP method (pH 1.2, 75 rpm) was used, both TMP and SMX in test and reference products dissolved more than 85% of the labeled amount of the drug in 15 min, the profile comparison with and $f_1$ and $f_2$ tests was, thus, unnecessary. Although the dissolution profiles of

**A.**




**B**

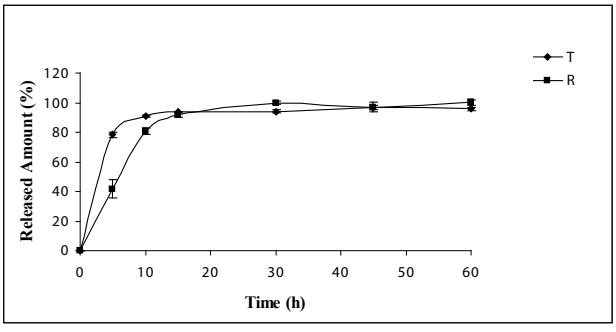



**Figure 3.** Mean (± SD) in vitro dissolution profiles of TMP (A) and SMX (B) from test (T) and reference (R) products. Dissolution studies were performed in 0.1 N HCl (900 mL) with paddle rotation speed of 75 rpm.

TMP obtained for test and reference products at pH 1.2 medium (50 rpm) were similar, based on $f_1$ and $f_2$ values, the dissolution profiles of TMP were dissimilar for other conditions (pH 4.6 and 6.8 with 50 rpm). In the case of SMX, for all three dissolution media given above, all dissolution profiles obtained for test and reference formulations were different. All these results indicate that dissolution studies are not sufficient to demonstrate the similarities of these tablet formulations, and that further in vivo studies are required.

## REFERENCES

1. Guidance for Industry: Dissolution testing of immediate release solid dosage forms. Food and Drug Administration, CDER, 1997. Retrieved from www.fda.gov/cder/guidance/index.htm
2. EMEA: CPMP Note for guidance on the investigation of bioavailability and bioequivalence, CPMP, July 2001.
3. Food and Drug Administration, Guidance for Industry: Waiver of *in vivo* bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system, Food and Drug Administration, Rockville, MD, 2000. Retrieved from www.fda.gov/cder/guidance/index.htm.
4. Martindale, The Complete Drug Reference. Thirty-third Edition. 2002. London S.C. Sweetman (Ed.), Pharmaceutical Press.
5. Avgerinos A., Athanasiou G., Malamataris S., Rapid simultaneous determination of trimethoprim sulphametoxazole and acetylsulphametoxazole in human plasma and urine by high-performance liquid chromatography. *J. Pharm. Biomed. Analysis,* 9(6): 507-510, 1991.
6. Garg S.K., Ghosm S.S., Mathur V.S. Comparative pharmacokinetic study of four different sulfanamides in combination with trimethoprim in human volunteers. *Int. J. Clin. Ther. Toxicol.,* 24(1): 23-25, 1986.
7. Patel R.B., Welling P.G. Clinical pharmacokinetics of co-trimoxazole (trimethoprim-sulphamethoxazole). *Clin. Pharmacokinet.,* 5: 405-423, 1980.
8. Petri W.A. Jr. Sulfonamides, trimethoprim-sulfamethoxazole, quinolones, and agents for urinary tract infections. Chapter 43. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 8th edition. New York, 2006. A.G. Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.) The McGraw-Hill.

9. Amidon G.L. Lennernas H., Shah V.P. Crison J.R. A theoretical basis for a biopharmaceutics drug classification: the correlation on an in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.,* 12, 413-420, 1995.
10. Wu C-Y., Benet L.Z. Predicting drug disposition via application of BCS: Transport/ absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm. Res.,* 22 (1):11-23, 2005.
11. The United States Pharmacopeia XXII, Dissolution <711>, 1990, p 1294.
12. FDA, Guidance for industry. Q2B Validation of analytical procedures: methodology, US department of healthy, food and drug administration, center for drug evaluation and research, 2001.
13. Liberman H.A., Lachman L., Schwatz J.B. Pharmaceutical Dosage Forms: Tablets. Second Edition, Revised and Expanded. Marcel and Dekker Inc., New York and Basel, 1990.
14. Florence A. T., Halbert G. W. Formulation. In: Comparative medicinal chemistry: The rational design, mechanistic study and therapeutic application of chemical compound. Hansch, C., Sammes, P. G. and Taylor, J. B. (Eds). 1 st Edition. Pergamon Press Plc; 1990. Vol. 6. p. 567-592. Oxford , UK .
15. Siewert M., Dressman, J., Brown C. K., Shah V. P. FIP/AAPS Guidelines to dissolution/ *in vitro* release testing of novel/special dosage forms. AAPS PharmSciTech, 4 (1) Article 7 (www. pharmscitech.org), 2003.
16. Sayar E. Jenerik Bir Trimetoprim ve Sülfametoksazol Tablet Üzerinde BSS ve Biyoeşdeğerlik Çalışmaları. Hacettepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Biyofarmasötik ve Farmakokinetik Programı, Doktora Tezi, Ankara, 2005.