



Estimating the Rate of Azithromycin Degradation Due to Increasing Temperature for Three Drug types by Spectrophotometer (UV) and Gas Chromatography-Mass Spectrometry (GC-MS)

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ABSTRACT

Azithromycin is a board antibiotic that affects various *Gram-positive* and *Gram-negative* bacteria, so azithromycin is used for the treatment different of bacterial infections, as well as used azithromycin as a prophylactics antibiotic after different surgeries. Azithromycin is used for children and adults, so it is available in pharmacies in different dosage forms like capsules, tablets, powder for reconstituting for oral administration. The aims of the present research is to assess the azithromycin stability from different available dosage forms (bioequivalence study) against temperature in hot climate country (Saudi Arabi). Three samples in the form of three drugs in which Azithromycin acts as an active ingredient were prepared and exposed to heat. These drugs are Azithromycin[®] 250 mg, Az-1[®] 250 mg, and Zirox[®] 250 mg. Three spectral techniques were used to study the change in concentration and chemical composition when the temperature is raised from 27°C to 60°C. The spectrometers used are ultraviolet Spectrometer and Gas Chromatography and Mass Spectrometry. The temperature of the three drugs was raised from 27°C to 60°C inside the water path. The ultraviolet spectrometer shows considerable degradation in Azithromycin concentration but the other two drugs are not affected appreciably by heating. The results obtained using the retention time technique of gas chromatography, show a change of the retention time to be (20.308-20.396-20.350) for Azithromycin[®], Az-1[®], and Zirox[®] scanned, respectively. This change may result from the difference in the matrix chemical composition of each drug. The mass spectrometry results show that rising temperature to 60°C the chemical bonds of the active ingredient to be decomposed to five compounds having m/z (43-72-99-158-198), respectively.

Keywords: Azithromycin, Chemical stability, Temperature degradation, Bioequivalence study, Gas Chromatography and Mass Spectrometry.

INTRODUCTION

Azithromycin is a board antibiotic that affects of three various *Gram-positive* and *Gram-negative*

bacteria, so the azithromycin used for the treatment different of bacterial infections as middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. In addition, azithromycin



effective against many sexually transmitted infections as including chlamydia and gonorrhea infections. Azithromycin is one of the medicine that can be used for kids, so it is prepared as a powder for reconstituting, in addition to capsule and tablets dosage form, usually, azithromycin dosage regiment as once daily so many doctors and clinics advised it in many bacterial infection states as well as prophylactics antibiotics after surgery¹.

Chemically, Azithromycin [9-deoxy-9a-aza-9a-methyl-9a-homoerythromycin] belongs to the azalide subclass of the macrolides group (see Fig. 1), which contains a 15-membered ring, with methyl-substituted nitrogen instead of a carbonyl group at the 9a position on the aglycone ring, which allows for the prevention of its metabolism^{1,2}.

Pharmacologically, the mechanism of action is interacting with bacteria protein synthesis, leading to prevents of bacteria growth. Also, inhibits mRNA translation³⁻⁵. Macrolides (Azithromycin belong family) drugs are active against bacteria and reinforce the immunity system⁶. So, the maintenance treatment with macrolides is a choice in the treatment of cystic fibrosis, bronchiectasis besides diffuse parochialities⁷⁻¹².

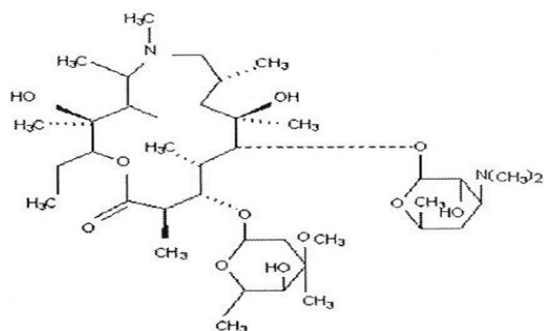


Fig. 1. Chemical structure of the azithromycin

Azithromycin is used in chronic obstructive pulmonary disease and neutrophilic airway disease¹²⁻¹⁴. In U.S.A azithromycin is an essential antibiotic¹². The chemical composition and structure of azithromycin can be determined using liquid chromatography^{16,17}. Some researchers use other techniques like mass spectrometer^{18,19} or differential poise ultra-metric device, for analysis of azithromycin²⁰⁻²². Also, amperometry device²³ or diffuse reflectance meter, infrared spectrometry is not analytically useful^{24,25}. There are other many

spectrophotometric techniques based on the visible spectrum that can also be used in Azithromycin studies^{26,27}. Fortunately, recent spectral techniques have proved to be more accurate, fast, and of relatively low cost²⁸⁻³⁰.

Methodology Study Design

The present study is a bioequivalence study, that investigates the stability of different three azithromycin dosage forms (Azithromycin[®] 250 mg, Az-1[®] 250 mg, and Zirox[®] 250 mg) against the raised in temperature by chemical analysis of active ingredient concentration of each forms using Gas chromatography-mass spectrophotometric instruments.

Source of Samples

The samples were collected from three different bioequivalence dosages form Azithromycin[®] 250 mg, Az-1[®] 250 mg, and Zirox[®] 250 mg.

The present study has taken into consideration to grouped the same batch from each dosage form for methodology.

Samples Collection

The present study focused on three different azithromycin dosage forms that available in Saudi Arabia Pharmacy, the following: Azithromycin[®] 250 mg, Az-1[®] 250 mg, and Zirox[®] 250 mg, the sample collected from each dosage forms after exposure raised in temperature.

Instruments

A Shimadzu GC-MS/MS (TQ8040) was used with capillary column (30 cm x 0.25 mm x 0.25 μm), (5% phenyl-95% dimethyl) (RTX-5), carrier gas helium (99.999% purity), constant flow 1.22 mL/min, temperature program 50:1°/min to 120:10°/min to 200:10°/min, 280:10°/min final hold (22), Ion Source Temp 200°C, Interface Temp 250°C.

A Shimadzu UV-1800 Series technique was employed having a wavelength range of 200-400 nm, having also light source change wavelength (340.8 nm).

UV Analysis

Sample's Dilution: 0.5 g of each sample was weighed and put into a 100 mL volumetric flask.

The volume was completed to 100 mL of isopropanol solution (A). 0.5 mL of this solution was transferred into a 50 mL volumetric flask and again diluted up to the mark of the flask using the isopropanol solution (B). Scanning and read of the absorption in UV device was done for all samples.

Exposure to Temperature: the amount of solution (B) for each sample was kept at 60°C for one hour than reading using UV device was made.

For each sample, the amount of sample solution was injected into the UV device. The absorbance and wavelength was them measured. A relation between absorbance and wavelength was displayed graphically (Table 1).

Table 1: Peaks of Azithromycin samples using UV-spectrophotometric between 200-400 nm

No	Sample	Wavelength nm	Absorbance
1	Azithrocin 250 mg	251.8	3.5
2	Azithrocin 250 mg using temp 60°C	251.8	1.8
3	Az-1 250 mg	252.2	2.0
4	Az-1 250 mg using temp 60°C	251.6	1.9
5	Zirox 250 mg	251.4	2.0
6	Zirox 250 mg using temp 60°C	251.4	1.9

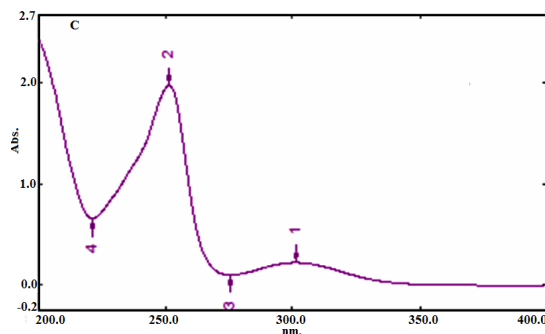
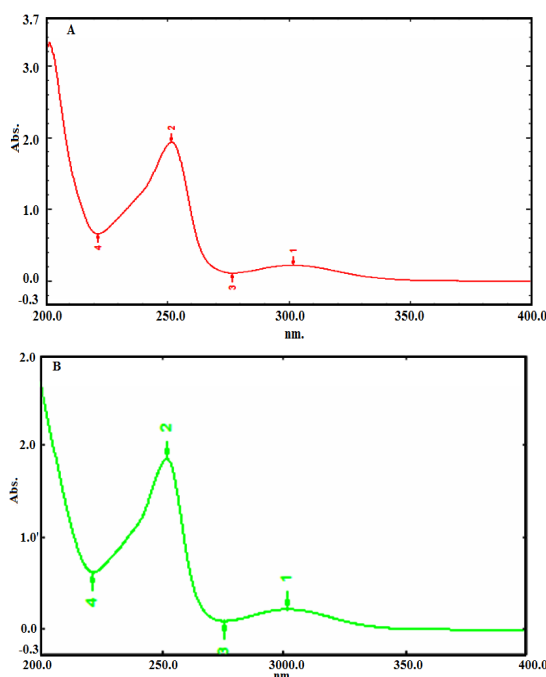


Fig. 2. UV spectrophotometric scanning between 200-400 nm for Azithrocin (A), Az-1 (B), Zirox (C) samples at 60°C temperature

GC-MS analysis

Sample's processing: A 0.01 g from each capsule were weighed and placed in a 10 mL volumetric flask, then 10 mL of methyl alcohol (HPLC grade) was added to each sample. The contents of each sample were mixed by a magnetic stirrer. The mixture was filtered, then the filtrate was subjected to GC-MS analysis. For each sample, 1 μ l was injected and a chromatography run was made. The chromatogram data was scanned and recorded in Fig. 2. The retention time was measured for each sample and compared with the library. The retention times (Rt) were measured for each sample and their results are given in Table (2).

Table 2: Relation time (Rt) and Mass per Atomic Number M/z of caffeine in different azithromycin samples

No	Sample	Rt	M/z
1	Azithromycin 250 mg	20.308	43-72-99-158-198
2	AZ-1 250 mg	20.396	43-72-99-158-198
3	Zirox 250 mg	20.350	43-72-99-158-198

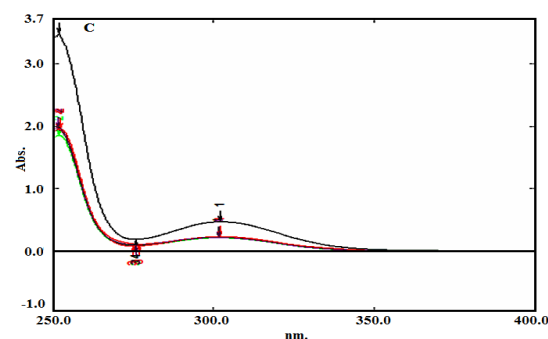


Fig. 3. UV spectrophotometric scanning between 200-400 nm for Azithrocin, Az-1, Zirox samples at room temperature

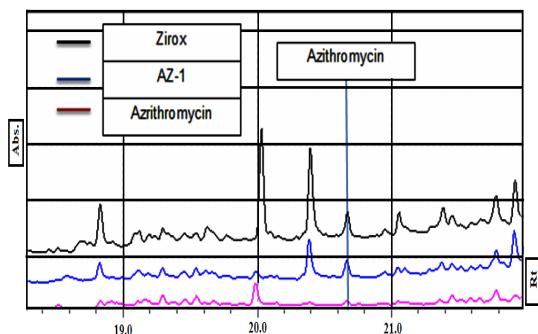


Fig. 4. Retention time versus absorption for Azithromycin, Az-1, and Zirox

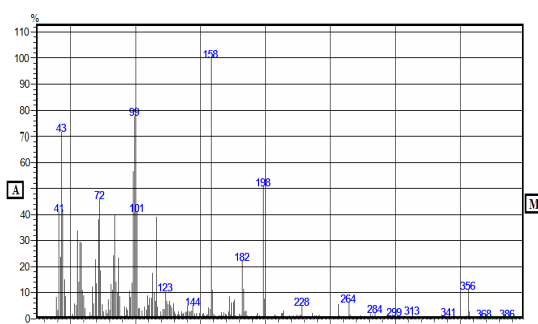


Fig. 5. Mass spectrophotometric: Molecular Weight (M) versus absorbance(A%)

RESULTS AND DISCUSSION

In this work, the effect of heating and raising of temperature on the active ingredient for three drugs has been studied. These drugs are Azithromycin 250 mg, Az-1 250 mg, and Zirox 250 mg. The chemical analysis was achieved by using UV-spectrometer, Gas chromatography (GC), and mass spectrometry (MS). The Fig. 2-5 and Tables (1,2) show the results.

In view of Fig. 2 beside Table (1), the UV spectrum shows a significant change in abundance (concentration) for azithromycin, where Table (1) shows that the absorption rate for wavelength 251.8 nm 3.5 at room temperature (27°C) is decreasing to 1.8 at 60°C. This means the raising temperature to 60 causes considerable degradation of azithromycin. However, for the other two drugs, Az-1 250 mg and Zirox 250 mg, Fig. 2-5 indicate the azithromycin is almost unaffected by raising the temperature from 27°C to 60°C.

Combined Gas chromatography and

mass spectrometer (GC-MS) spectra for the three samples, shows the heating and raising in temperature degree of the three samples causes a considerable change in both absorption rate and chemical composition. The absorption rate versus retention time for azithromycin shows a very low absorption rate at 60°C compared to Az-1 250 mg and Zirox 250 mg. The mass spectrometry result for m/z in the table (B) shows the azithromycin is fragmenting to separate compounds that having m/z values (43,72,99,158,198).

So, must be store the azithromycin-containing dosage form, at a temperature below 27°C, to save their activity. The other two samples resist heating up to 60°C.

The gas chromatography shows a change in the retention time of azithromycin in the three drugs, which assumes the values (20.308, 20.396, 20.350) for, Az-1 and Zirox, respectively. This may be related to the matrix change of the drugs.

CONCLUSION

There are a significant impact of heating and raising of temperature degree in the stability of azithromycin 250 mg, when a raising the temperature from 27°C to 60°C, but not impacting on Az-1 250 mg and Zirox 250 mg dosage forms. This effect resulting in the degradation of the active ingredient (azithromycin) via the destruction of chemical bonds and produce of five different compounds (fragmentation). This fragmentation is observed for Az-1 and Zirox.

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Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

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