
ORIGINAL ARTICLE

DEGRADATION STUDIES OF AZITHROMYCIN AND ITS SPECTROPHOTOMETRIC DETERMINATION IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, accurate and rapid spectrophotometric method for the estimation of azithromycin has been developed by the acidic hydrolysis of the drug with sulfuric acid and monitoring the absorbance at 482nm. All variables affecting the reaction conditions such as sulfuric acid concentration, heating time, temperature and dilution solvents were carefully studied. Analytical parameters such as stability, selectivity, accuracy and precision have been established for the method and evaluated statistically to assess the application of the method. The method was applied successfully for the assay of azithromycin dihydrate in pure and pharmaceutical dosage forms as tablets, capsules and suspensions. The method was found to have the advantages for simplicity, stability, sensitivity, reproducibility and accuracy for using as an alternate to the existing non-spectrophotometric methods for the routine analysis of the drug in pharmaceutical formulations and also in pharmaceutical investigations involving azithromycin dihydrate.

Keywords: Azithromycin; degradation; spectrophotometry; pharmaceutical analysis.

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INTRODUCTION

Azithromycin, (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin A) dehydrate, is an azalide, a subclass of macrolide antibiotics, for oral administration (figure 1). Azithromycin is derived from erythromycin; however it differs chemically from erythromycin in that a methyl substituted nitrogen atom is incorporated into the lactone ring and semisynthetic erythromycin derivative (The United States Pharmacopoeia). It exhibits a more extensive spectrum of activity, greater acid stability, better oral bioavailability and more favorable pharmacokinetic behavior than erythromycin (Lode *et al.*, 1996). Its unique pharmacokinetic properties include extensive tissue distribution and high drug concentrations within cells. The most innovative feature is the efficacy and safety of a 3-day oral regimen (Dunn and Barradell, 1996). Azithromycin plays a leading role in the treatment of respiratory tract infections, toxoplasmosis, nonclassical pathogens such as *Helicobacter Pylori*, pediatric infections and opportunistic infections in AIDS.

Azithromycin has been analyzed in biological samples by microbiological method [Runes HR, Roncari AFF. (2005)] and high performance liquid chromatography using electrochemical detector (Carmen *et al.*, 2001), Kees *et al.* (1998), Nigovic and Imunic (2003), Taninaka *et al.* (2000) and fluorescence (Tod *et al.*, 1992). However, there is noticeable shortage of methods described in the literature for its determination in pharmaceutical dosage forms. Chromatographic methods developed for azithromycin quantitation (Kanfer *et al.*, 1998), Kamau *et al.* (2002), Horie *et al.* (1998) demand expensive equipment and could not be available in many laboratories. One electrochemical method has been described in literature (Gandhi *et al.*, 2000), but it is based on reduction of azithromycin in strongly basic media at mercury electrode. The degradative behavior and determination of azithromycin by spectrophotometery has not been reported.

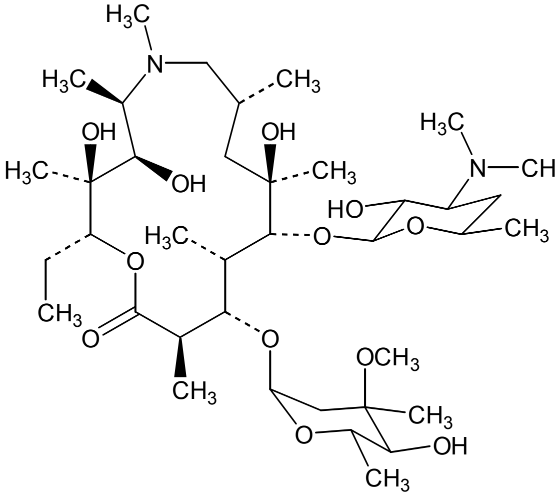

Fig. 1

The aim of the present study has been to examine the degradative properties and assay of azithromycin by spectrophotometer. A colorimetric procedure for the determination of azithromycin in its pharmaceutical formulations was optimized and effect of temperature and time was studied. The following research work is based on the observation that macrolide antibiotics like erythromycin undergo hydrolytic cleavage at glycosidic linkage when reacted with 27 N sulfuric acid and produce aglycone moiety that is erythronolide that exhibits strong absorption in visible region at 482 nm. It has been assumed that azithromycin, an azalide, having similar structure as if erythromycin will undergo the same degradation when treated with strong oxidizing agents. Similarly, when macrolide antibiotics reacted with strong base, the lactone bond was saponified.

EXPERIMENTAL

Materials and instrument

Azithromycin dihydrate (Pharmaceutical grade) was supplied by Platinum Pharmaceuticals Pvt. Ltd. Karachi, Pakistan, and was used without any further purification. All reagents were of analytical grade (Merck GmbH, Germany). Shimadzu 1601 UV visible spectrophotometer with quartz cells of 10-mm path length

Stock solutions of (39.25 $\mu g\ mL^{-1}$) azithromycin dihydrate prepared by dissolving 7.85 mg of azithromycin dihydrate in 100 ml of methanol using a bath sonicator and diluting 5 ml of this solution with 5 ml of sulfuric acid (27 N).

Same procedure was repeated for stock solutions in hydrochloric acid (11N), phosphoric acid (5 N) nitric acid (16 N), sodium hydroxide (0.2 N), potassium hydroxide (0.4 N) and ammonia (15 N).

Preparation of calibration curve for acids

Suitable aliquots (1–10 ml) of the stock solution were pipetted into 10 ml volumetric flasks and the volume was made upto mark with 27 N sulfuric acid. The solutions were refluxed for 30 minutes in a water bath at 60°C and absorbance was measured at 482 nm against reagent blank.

The above procedure was repeated six times and similar procedure was adopted for hydrochloric acid (11N), phosphoric acid (5 N) and nitric acid (16 N), which gave strong absorption in visible region and the maxima appeared at 482 nm for phosphoric acid, at 483 nm for hydrochloric and 483 nm for nitric acid. Mean absorbance values along with the regressed values (method of least squares) and statistical data for these acids are shown in table 1.

Table 1: Mean[a] absorbance and statistical data for the estimation of azithromycin in acidic and alkaline media

Concentration	<—————— Medium ——————>						
($M\times10^{-4}$)	H_2SO_4	H_3PO_4	HCl	HNO_3	NaOH	KOH	NH_3
0.20	0.08	0.04	0.05	0.04	0.12	0.15	0.17
0.40	0.18	0.09	0.10	0.09	0.23	0.30	0.35
0.60	0.29	0.15	0.16	0.13	0.35	0.45	0.55
0.80	0.39	0.22	0.21	0.17	0.45	0.60	0.74
1.00	0.48	0.28	0.27	0.21	0.55	0.75	0.93
1.20	0.58	0.35	0.32	0.26	0.68	0.93	1.12
1.40	0.67	0.41	0.37	0.30	0.79	1.07	1.27
1.60	0.77	0.46	0.45	0.34	0.87	1.23	1.47
1.80	0.87	0.52	0.49	0.39	0.97	1.36	1.66
2.00	0.99	0.59	0.54	0.43	1.10	1.51	1.86
Correlation coefficient	0.99976	0.99952	0.99921	0.99973	0.99940	0.99976	0.99980
Standard Error	0.00561	0.00418	0.00481	0.00219	0.00820	0.00730	0.00821
Intercept	1.54×10^{-2}	2.74×10^{-2}	8.00×10^{-3}	6.67×10^{-5}	1.94×10^{-2}	3.33×10^{-3}	1.33×10^{-2}
Slope	4.96×10^{-1}	3.08×10^{-1}	2.76×10^{-1}	2.15×10^{-1}	5.38×10^{-1}	7.62×10^{-1}	9.32×10^{-1}

[a]Mean of six values.

Table 2: Effect of temperatures on azithromycin degradation after heating for 30 minutes

Temperature °C	<—————— % Drug recovery ——————>						
°C	H_2SO_4	H_3PO_4	HCl	HNO_3	NaOH	KOH	NH_3
33	92.38	38.18	38.16	71.26	79.77	91.34	2.68
40	100.11	46.93	80.63	100.00	81.11	96.04	21.36
50	95.63	85.29	100.00	43.93	83.15	96.37	56.46
60	85.43	100.00	90.22	--	84.22	97.90	83.02
70	83.86	99.81	83.17	--	88.24	99.53	97.50
80	83.52	96.46	62.82	--	100.00	100.00	100.00
ε ($mole^{-1}L\ cm^{-1}$)	4460	2685	2555	2140	5541	5543	9275

For bases

Suitable aliquots (1–10 ml) of the stock solution were pipetted into 10 ml volumetric flasks and the volume was made upto mark with 0.2 N sodium hydroxide. The solutions were refluxed for 30 minutes in a water bath having 60 °C temperature and absorbance measured at 215 nm.

The above procedure was repeated six times, similar procedure was adopted for, potassium hydroxide and ammonia, which exhibit strong absorption in ultraviolet region and the wavelength of the maximum absorption appeared at 217 nm for potassium hydroxide and at 216 nm for ammonia. Mean absorbance values along with the regressed values (method of least squares) and statistical data for these bases are shown in table 2.

RESULTS AND DISCUSSION

It have been reported that incorporation of weakly basic tertiary amine nitrogen function into the macrolide ring increases the stability of azithromycin to acid catalyzed degradation. It also increases the lipid solubility of the molecule thereby conferring unique pharmacokinetic and microbiological properties. Just like other macrolide antibiotics, azithromycin contain a large lactone ring glycosidically linked with the nitrogenous sugar moieties. The lactone ring structure provides a base for conducting degradative studies of drug.

Azithromycin is spectrophotometricaly inactive because of its chemical structure and does not give any absorption in the UV/visible region. Previously it had been reported that erythromycin when reacted with 27 N sulfuric acid, undergoes hydrolytic cleavage at glycosidic linkage and produce degradative product, erythronolide, that gives absorbance maxima at 482 nm and the same reaction formed the basis of the further work. This research study is based on the degradation of azithromycin in acidic and alkaline media and the degradative product gave strong absorption in UV/visible region. Optimum conditions for degradation involving optimum temperature, time and acid/base concentration were also determined using sulfuric, hydrochloric, phosphoric, nitric acid and sodium hydroxide, potassium hydroxide and ammonia solution respectively.

Table 3: Effect of time interval on azithromycin degradation at optimum temperature

↓Time(min)	<——— % Drug recovered ———>						
	H_2SO_4	H_3PO_4	HCl	HNO_3	NaOH	KOH	NH_3
Temp(°C) →	40	60	50	40	80	80	80
0	56.14	00.00	8.57	5.33	100.41	100.08	100.11
5	90.04	17.80	29.52	39.07	76.77	79.06	41.24
10	95.75	49.44	60.00	66.98	75.66	77.96	25.07
15	96.21	65.04	78.15	80.47	74.80	76.76	16.77
20	96.45	81.33	87.78	85.12	74.32	75.79	11.92
25	98.11	87.19	92.96	92.33	74.17	74.98	8.99
30	98.26	93.48	95.19	96.74	73.71	73.65	7.47
35	98.39	96.19	97.41	99.30	73.02	73.30	6.28
40	98.59	99.67	97.59	99.77	72.16	73.22	5.41
45	98.93	99.74	98.70	100.00	71.56	72.98	5.14
50	99.57	99.81	99.63	100.23	70.99	72.80	4.55
55	99.78	99.83	100.19	100.23	70.87	72.69	4.27
60	100.00	99.83	100.56	100.47	70.68	72.53	4.04
ε (mole^{-1}L cm^{-1})	4600	2700	2700	2150	4100	6350	9300

It was evident from these studies that change of acid plays a significant role in deviation of absorption values. With sulfuric acid, maximum absorption was observed as compared to hydrochloric, phosphoric and nitric acid. Thus sulfuric acid is considered as standard for conducting azithromycin degradation studies. A drastic change was also observed in the molar absorptivity of the drug with the change of acid, used for degradation studies; thus sulfuric acid gave maximum molar absorptivity as compared to other acids.

Consequently, in the spectrophotometric assay of azithromycin, measurement of absorption maxima was recorded at 482 nm for sulfuric and phosphoric acid and at 483 nm for hydrochloric and nitric acid. Measurement of these absorbance bands have been employed for the assay of azithromycin. Beer's law was found to be obeyed between 0.2 to 0.02 mMole for sulfuric, hydrochloric, phosphoric and nitric acids. Correlation coefficients for sulfuric, phosphoric, hydrochloric, and nitric acids were found to be 0.99976, 0.99952, 0.99921 and 0.99973 respectively, signifying that a linear relation existed between absorbance and concentration of the drug. Regression analysis was performed on the experimental data. The raw data along with the results of regression analysis (method of least squares) is given in table 1 for acids. Regression equations for sulfuric, phosphoric, hydrochloric and nitric acids were $y=0.496x+0.0154$, $y=0.308x+0.0274$, $y=0.276x+0.0080$ and $y=0.496x+0.000667$ respectively. These low values indicate the closeness of the experimental points to the least squares line. The fact is in concurrence with the low values of the standard error of the mean (S.E.M.) absorbance of the solutions used for preparing the calibration curve.

The similar procedure was repeated in alkaline media with sodium hydroxide (0.2 N), potassium hydroxide (0.4 N), and ammonia solution (15 N), and calibration curve were plotted. During these studies wavelength of maximum absorption was observed 215 nm 217 nm and 216 nm for sodium hydroxide, potassium hydroxide and ammonia solution respectively. Beer's law was found to be obeyed between 0.2 to 0.02 mMole for all thee mediums. Correlation coefficients for sodium hydroxide, potassium hydroxide and ammonia solution were found to be 0.99940, 0.99976, and 0.99980 respectively, signifying that a linear relation existed between absorbance and concentration of the drug. Regression analysis was performed on the experimental data. The raw data along with the results of regression analysis (method of least squares) is shown in table 1 for bases. Regression equations for sodium hydroxide, potassium hydroxide and ammonia solution were $y = 0.538x + 0.0194$, $y = 0.762x + 0.00333$ and $y = 0.932x + 0.0133$ respectively. These low values indicate the closeness of the experimental points to the least squares line. The fact is in concurrence with the low values of the standard error of the mean (S.E.M.) absorbance of the solutions used for preparing the calibration curve.

Effect of temperature on azithromycin degradation

It has been observed that the rate of azithromycin degradation in acidic medium was strongly affected by change in temperature. For this purpose 5 ml of each 0.2 mMole azithromycin and sulfuric acid (27 N) were reacted at room temperature and at 40, 50, 60, 70 and 80°C for 30 minutes on water bath and then cooled. Absorbance of each solution was measured at 482 nm.

It has been observed that initially there was an increase in absorbance with increasing temperature that gradually decreased as temperature increased to 80°C. The percentage drug recovered at each temperature was calculated and graphs were plotted between temperatures against % drug recovered. The temperature at which 100% drug recovered was considered as optimum temperature. Results of these studies are shown in table 2.

In case of sulfuric and nitric acid, being stronger acid and also strong oxidative agents, 100% drug recovery was achieved at 40°C which was considered as the optimum temperature for both acids. In case of hydrochloric acid, the optimum temperature was 50°C and for phosphoric acid it was 60°C. It was further observed that in case of sulfuric, hydrochloric and nitric acid, the % age drug recovery decreased gradually as the temperature was increased beyond optimum temperature to 80°C. In case of nitric acid, there was decrease in % drug available at 50°C that diminished completely when the temperature was further increased.

Same procedure was repeated with sodium hydroxide 0.2 N, potassium hydroxide 0.4 N and ammonia solutions 15 N and % age drug recovery was calculated for each base. It was observed that 100% drug was available at 80°C with each reactant. It was also observed that in case of sodium hydroxide and potassium hydroxide there was only slight increase in the percentage drug recovery when the temperature varied from room temperature to 80°C. In case of ammonia there was a drastic increase in the % drug recovery up to 60°C after which the increase was slow to 80°C. The results of these studies are given in table 2.

Effect of time on azithromycin degradation

Like temperature, the rate of azithromycin degradation was dependent on time interval. For this purpose 30 ml working solution each of azithromycin and sulfuric acid 27N were mixed and heated at 40ºC (optimum temperature) previously maintained on water bath and readings were noted at 0 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 minutes time interval. Absorbance was measured at 482 nm against reagent blank. The % drug recovery was calculated at each time interval and graph was plotted between time interval and % drug available. The same procedure was repeated with hydrochloric, phosphoric and nitric acid. It has been observed that as the time interval increased, the absorbance also increased gradually. The time at which there was 100 % drug recovery was considered as optimum time for each acid.

It was observed that >93% drug recovered within 60 minutes with each acid. In case of sulfuric acid, 90% of drug was recovered within 5 minutes, which increased to 100% after 60 minutes. These studies were carried out at (optimum temperature) 40°C for sulfuric and nitric acid, at 50°C and 60°C for hydrochloric and phosphoric acid respectively. The results of this study are shown in table 3. The above procedure was repeated for sodium hydroxide, potassium hydroxide and ammonia solution and the absorption was measured in the range of 215-217 nm. It has been observed that 100% drug was available at 0 time which decreased gradually with the passage of time until it become constant. In case of ammonia solution the decrease in % drug recovery was rapid as compared to sodium hydroxide and potassium hydroxide. The results are shown in table 3.

CONCLUSION

Azithromycin can be estimated using both the acids and bases at 482±1 nm and 215±1 nm, respectively. Both methods have the advantages of simplicity, sensitivity, reproducibility and accuracy. However, sulfuric acid can be employed as standard reagent for carrying out degradation of azithromycin and subsequently be analyzed by colorimetric measurement.

Results of the above studies indicate the suitability of the methods to quantitate azithromycin in bulk as well as in formulations. The developed methods are comparable to the official method elaborated in the United States Pharmacopoeia. They may also be selected as alternate to the existing, time-consuming and expensive methods like voltammetric assay and high performance liquid chromatography (HPLC) with electrochemical detector.

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