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RESEARCH ARTICLE

Development of Rapid, Simple and Stability-Indicating Method for Determination of Azithromycin Using RP-HPLC

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ABSTRACT:
An accurate, sensitive, precise and stability indicating (in accordance with ICH guidelines) reversed phase high performance liquid chromatographic (RP-HPLC) method of analysis of Azithromycin was developed and validated. The chromatographic conditions comprised of a reversible phase C8 column (250 × 4.6 mm, 5µ) with a mobile phase consisting of a mixture of dipotassium hydrogen Phosphate and Acetonitrile in the ratio of 65:35 at pH 6.5 adjusted with phosphoric acid. Flow rate was 1.5 ml/min, the detection was carried out at 200 nm and the retention time of Azithromycin was 2.99 min. Azithromycin was subjected to acid and alkali hydrolysis, oxidation and photochemical degradation. The method was validated for accuracy, precision and robustness. The results indicate that the drugs are susceptible to degradation in different conditions. All the peaks of degraded products were resolved from the active pharmaceutical ingredient with significant different retention times. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

KEY WORDS: RP-HPLC, Azithromycin, validation, degradation, stability indicating.

INTRODUCTION:
Azithromycin is a semi-synthetic macrolide antibiotic used clinically for a wide range of bacterial infections[1,2], and it is listed in the World Health Organization[3] where it is indicated for the management of atypical infections. Chemically it is (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-{[(2,6dideoxy-3-o-methyl-∞-L-ribo hexopyranosyl) oxy]-2- ethyl-3,4,10-tri hydroxyl-3,5,6,8,10,12,14-hepta methyl- 11-[(3,4,6-trideoxy-3-(dimethyl)amino)-β-D-xylo hexo pyranosyl)]oxy]-1-oxa-6-azacyclopentadecan15-one.[4] **(Fig. 1)** shows the structure of Azithromycin. Suitable analytical methods are required to control the quality of azithromycin (AZT) in bulk material, in-process control and finished product.

There were many published articles for the analysis of azithromycin such as biological methods[5], electroanalytical methods[6], spectrophotometric methods[7], capillary electrophoresis[8], thin layer chromatography[9] and liquid chromatography[10-13]. A review by Sharma and Mullangi of the analytical methods for azithromycin was reported[14].

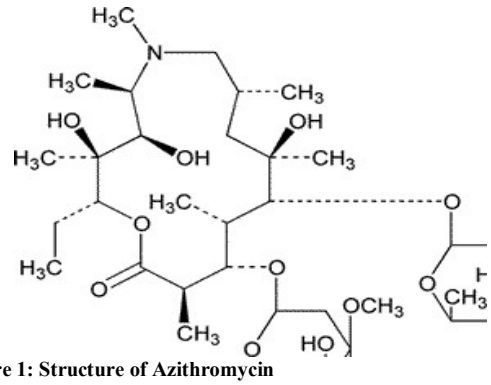

Figure 1: Structure of Azithromycin

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The aim of the present work is to develop simple, accurate, and stability indicating method for the determination of Azithromycin in the presence of its degradation products after performing stress studies under a variety of ICH recommended test conditions. However, the method does not discuss the identity of the degradation products, the present work only deals with method development.

EXPERIMENTAL:

Materials:

Azithromycin Dihydrate was supplied as gift sample from MEDICO Labs., Homs, Syria. All other reagents used were AR grade and HPLC grade. Acetonitrile (HPLC grade-Fischer Scientific), Methanol (HPLC grade-Fischer Scientific), Dipotassium hydrogen phosphate (Rankem), Ortho Phosphoric acid (Rankem) were used for analysis. Hydrochloric acid (Surechem, England), Sodium hydroxide, Hydrogen peroxide, Filters 0.45µm. Water for HPLC was distilled from glass apparatus.

Instrumentation:

The HPLC instrument used was Shimadzu LC-20AD system equipped with photodiode array (PDA) detector.

Chromatographic Conditions:

The mobile phase was prepared by mixing Acetonitrile, and phosphate buffer in the ratio of (35:65 v/v) and pH 6.5 adjusted with ortho phosphoric acid. It was filtered through 0.45 µ membrane filter. All determinations were performed at ambient temperature (25 ºC) using C8, (250×4.6 mm, 5 µm), reverse phase column (GL Science). The column effluent was monitored at 200 nm. The injection volume was 20 µl with a flow rate of 1.5 ml/min.

Standard Preparation:

Accurately weighed quantity, 500mg of Azithromycin was transferred into 100 ml of volumetric flask and adds 30 ml of (water: ACN, 30:70 v:v) and sonicate for 15 min make up the volume with the same solvent. Transferred above solution 5 ml into 50 ml volumetric flask and diluted to the mark. The resultant solutions were appropriately diluted to obtain final concentration in the range 50 - 150 µg/ml and chromatograms were run. The analysis was repeated in triplicate.

Stress degradation of Azithromycin:

Different kinds of stress conditions were employed on Azithromycin based on the guidance available from ICH Stability Guideline. The details of the stress conditions applied were as follows:

Preparation of acid and base induced degradation product:

Accurately weighed 500 mg of drug was dissolved in 100 ml of (water:ACN, 30:70 v:v). The drug was subjected to accelerated degradation under acidic and basic conditions by refluxing with 1 N HCl and 1 N NaOH, respectively, at 70 °C for a period of 1 hour. The accelerated degradation in acidic and basic media was performed in the dark in order to exclude the possible degradation effect of light on the drug. The resultant solutions were appropriately diluted and chromatograms were run.[15]

Preparation of hydrogen peroxide induced degradation product:

Accurately weighed 500 mg of drug was dissolved in 100 ml of methanol. Subsequently, 10 ml of hydrogen peroxide 30.0% v/v was added and the solution was heated in boiling water bath for 1 hour till the removal of excess hydrogen peroxide. The resultant solutions were appropriately diluted and chromatograms were run.[15]

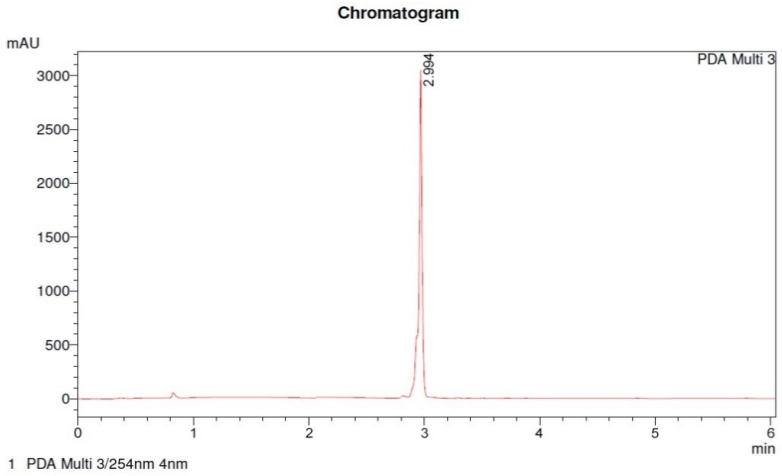

Figure 2: HPLC Chromatogram of Azithromycin

Photochemical degradation product:
Accurately weighed 500 mg of drug was dissolved in 100 ml of methanol. The photochemical stability of the drug was also studied by exposing the drug solution to direct sunlight for 24 h. The resultant solutions were appropriately diluted and chromatograms were run.[15]

RESULTS AND DISCUSSION:

HPLC analysis:
The chromatographic conditions were optimized with a view to develop a validated method for the determination of azithromycin and a stability indicating assay method. As showed in **(Fig. 2)**, the experimental studies revealed that the column, RP-C8 (250 × 4.6 mm, 5µ) was most suitable, since it produced best chromatographic performance and acceptable peak characteristics including high resolution and very good sensitivity without no interferes, impurities, or tailing in the peak.

Analytical Method Validation:
Method validation was performed under a variety of ICH and United States Pharmacopeia 30 recommended test conditions.[13,15]

Linearity:
Five standard solutions of Azithromycin were prepared with the concentrations (50, 75, 100, 125 and 150 µg/ml), each solution was injected six times in HPLC. **(Fig. 3)** and **(Fig. 4)** show the linearity of Azithromycin with a correlation coefficient of 0.9997.

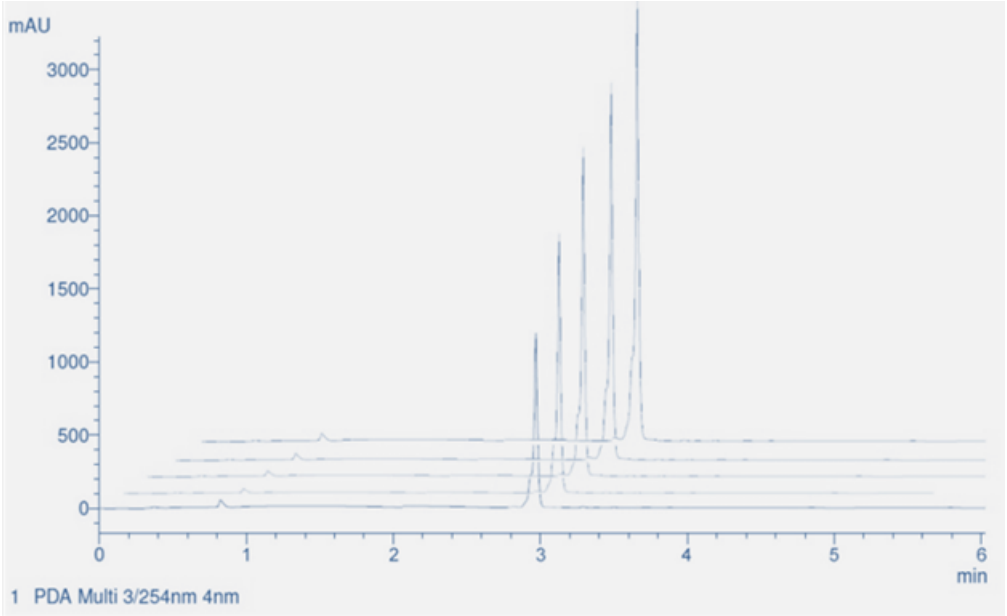

Figure 3: Chromatograms of linearity of Azithromycin

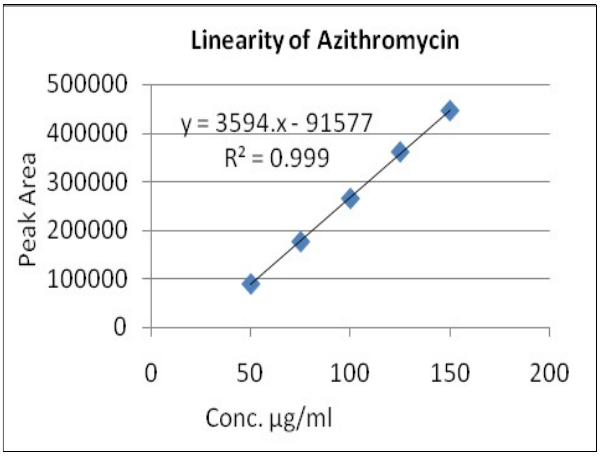

Figure 4: Linearity of Azithromycin

Range:
Linearity, precision and accuracy were conformed in the interval (50, 75, 100, 125 and 150 µg/ml) for Azithromycin.

Accuracy:
Concentrations of (50, 100, and 150 µg/ml) have been used to study the accuracy of Azithromycin (Table 1). Results indicate that the individual recovery of Azithromycin ranges from 99.23% to 100.87% with mean recovery of 99.85% and %RSD of 1.14%. The recovery of the Azithromycin by proposed method is satisfactory as %RSD is not more than +2.0% and mean recovery between 98.0 – 102.0%.

Table 1: Accuracy of Azithromycin

Conc. µg/ml	Area	Calc. Area	Calc. Conc.	Recovery %	AVR	SD	RSD %
50	88676	88676	50.15	100.30			
50	89558	89558	50.39	100.79			
50	90872	90872	50.76	101.52	100.87	0.614	0.609
100	268625	268625	100.22	100.22			
100	266734	266734	99.69	99.69			
100	259876	259876	97.78	97.78	99.23	1.280	1.290
150	443726	443726	148.93	99.29			
150	449878	449878	150.65	100.43			
150	440283	440283	147.98	98.65	99.46	0.901	0.906

Precision:

The solution 75 µg/ml has been injected ten times. Standard deviation and relative standard deviation of the response (peak area) have been calculated and results were illustrated in (Table 2). The RSD% for repeatability of sample preparation is 1.11 %, this shows that precision of the method is satisfactory as RSD% is not more than +2.0%.

Table 2: Precision of Azithromycin.

Conc. µg/ml	Area	AVR	STDV	RSD %
75	178647			
75	180983			
75	177836			
75	175364			
75	175553	177185.11	1970.86	1.11
75	174466			
75	176772			
75	177124			
75	177921			

Intermediate Precision:

The RSD% for intermediate Precision of sample preparation is 1.23%. (Table 3) shows that the intermediate Precision of the method is satisfactory as RSD% is not more than +2.0%.

Table 3: Intermediate Precision of Azithromycin.

No. Of injection	Conc. µg/ml	Area
Solution A		
1	75	177823
2	75	175529
3	75	176923
4	75	178225
5	75	179882
6	75	178293
Solution B		
1	75	180231
2	75	181723
3	75	180923
4	75	182992
5	75	179865
6	75	176752
Mean		**179096.75**
STDV		**2213.16**
RSD%		**1.23**

Robustness:

The method robustness and ruggedness was determined by analyzing same sample at normal operating conditions and also by changing some operating analytical conditions such as flow rate and wave length detection. As shown in (Table 4) and (Table 5), the slight changes in the method parameter do not affect the analysis.

Table 4: Flow rate modification of Azithromycin.

Modification of Flow Rate			
	Retention time (min)		**Rt of unchanged flow rate**
No. of Injection	**Flow Rate= 1.3 ml/min**	**Flow Rate= 1.7 ml/min**	**Flow Rate= 1.5 ml/min**
1	3.211	2.654	2.992
2	3.212	2.662	2.994
3	3.212	2.657	2.993
4	3.213	2.669	2.995
5	3.211	2.652	3.001
6	3.212	2.662	2.988
AVR	3.211	2.659	2.993
SD	0.00075	0.0062	0.0042
RSD%	0.023	0.235	0.142

Table 5: Wave length modification of Azithromycin.

Modification of Wave Length			
	Peak Area		**Peak Area of unchanged wave length**
No. of Injection	**λ=195 nm**	**λ=205 nm**	**λ=200 nm**
1	172361	176541	178647
2	171998	175239	180983
3	170927	176892	177836
4	171722	175331	175364
5	172174	174837	175553
6	172981	175227	174466
AVR	172027.16	175677.83	177141.5
SD	685.14	829.74	2463.62
RSD%	0.398	0.472	1.39

LOD and LOQ: The calculated LOD and LOQ were 7.65 µg/ml and 23.19 µg/ml respectively.

Stability indicating property:

The chromatogram of no stress treatment sample (as control) showed no additional peak (Fig. 3). The chromatogram of acid degraded sample showed one additional peak with retention time of 1.537 min, but it's

well resolved from the peak of azithromycin with a significant difference in the retention time between the two peaks (Fig. 5). The chromatogram of alkali degraded sample also showed one additional peak at retention times of 1.199 min and it's well resolved from the peak of azithromycin (Fig. 6). The chromatogram of H_2O_2 degraded sample showed an additional peak at 1.828 and it's well resolved from the peak of azithromycin (Fig. 7). There is no peak related to degradation in photo degraded sample of Azithromycin.

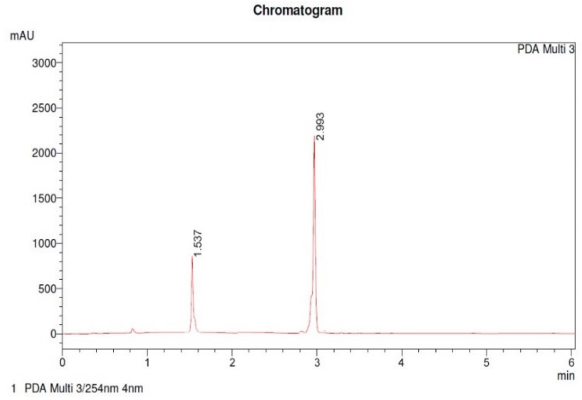

Figure 4: Chromatogram of Acid Degradation Azithromycin

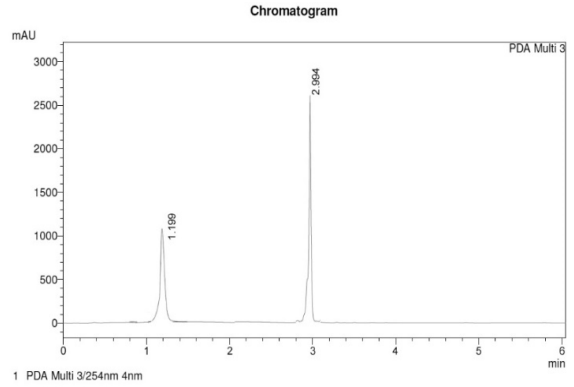

Figure 5: Chromatogram of Base Degradation Azithromycin

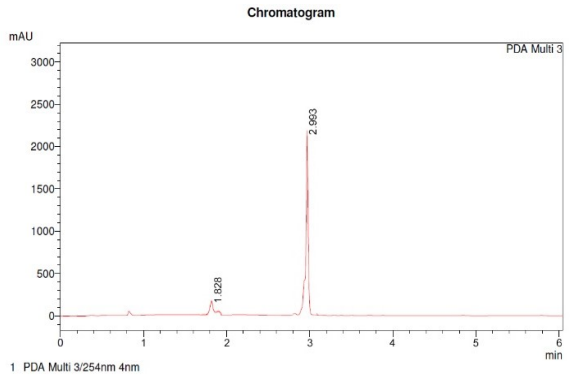

Figure 6: Chromatogram of H_2O_2 degradation Azithromycin

CONCLUSION:

The developed HPLC technique is precise, accurate and stability indicating for the analysis of Azithromycin in bulk and in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, under all stress conditions using HCl, NaOH, H_2O_2 and UV light, it can be employed as a stability indicating one.

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