RESEARCH ARTICLE

# Spectrophotometric Determination of Azithromycin Dihydrate in Formulation and its Application to Dissolution Studies

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## ABSTRACT

This article aims to provide a simple, precise, accurate, and sensitive UV spectrophotometric method to analyze azithromycin dihydrate formulations. Azithromycin dihydrate is a semisynthetic azalide subclass of macrolides antibiotic used to treat various bacterial infections. This method emphasizes the chemistry of azalide that azithromycin undergoes a hydrolytic reaction at glycosidic bond when reacted with concentrated sulphuric acid to produce yellow aglycone solution exhibiting absorbance maximum at 480 nm. Experimental conditions for the analytical method were optimized and validated. The calibration curve was linear in the range of 20 to 60 µg/mL with $R^2 = 0.9994$ with limit of detection (LoD), and limit of quantitation (LoQ) of 1.936 and 5.868 µg/mL, respectively. The developed analytical method met the ICH Q2 (R1) criteria and was successfully applied to azithromycin mucoadhesive buccal tablet, which showed no interference with the excipients.

**Keywords:** Azithromycin Dihydrate, Dissolution, Mucoadhesive buccal tablet, UV spectrophotometry,

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**Conflict of interest:** None

## INTRODUCTION

Azithromycin dihydrate {2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-11- [(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-2-ethyl-3,4,10-trihydroxy-13-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl] oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6 azacyclopentadecan-15-one;dihydrate} is a second generation azalide subclass of macrolide semi-synthetic antibiotic derived from Streptomyces (Figure 1).[1] Dr. Slobodan Dokic and his team discovered azithromycin in 1980 and Pliva patented it in 1981. With licensing agreement, Phizer brought it in the brand name of Zithromax in 1991.[2] Macrolide antibiotics have their structure-based names which explains the presence of macrocyclic lactone usually having 12 to 17 atoms, a ketone group, a neutral sugar linked to amino sugar or lactone ring the having presence of dimethylamino group functioning as a basicity group and salts formation responsible for any weak bond formation with the compounds. Azithromycin contains 15 membered lactone rings and is stable under acidic conditions because it does not form cyclic ketal.[3] Azithromycin inhibits bacterial protein synthesis, which binds and interferes with the protein assembly of the 50S large ribosomal subunit inhibiting the growth of the polypeptide chain. It binds at the polypeptide exit tunnel, close to the peptidyl transferase (PT) centre on the 23S rRNA but does not inhibit PT. The basicity of azithromycin enhances penetration to the outer membrane of the bacteria, which improvises bactericidal activity against gram-negative bacteria.[4] Azithromycin is available in the form of tablets,, oral suspension, intravenous solution, ophthalmic solution, powder for suspension, granules for suspension extended-release.[5,6] Azithromycin is known for using lower and upper respiratory tract infections like pneumonia, tonsillitis/pharyngitis, sinusitis, skin infections. It is more active than any other macrolides against influenzas but less active against

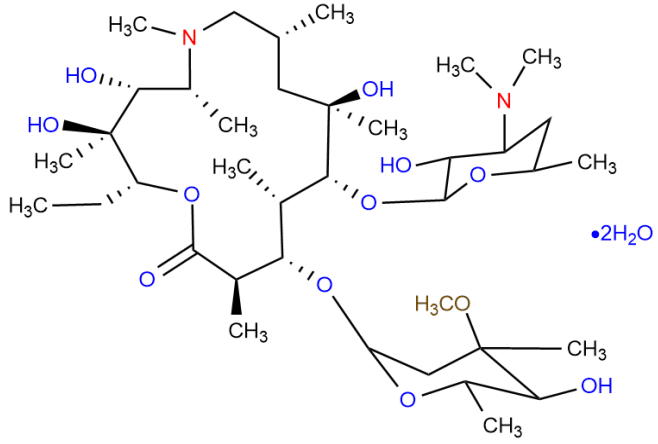


**Figure 1:** Azithromycin Dihydrate

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gram-negative cocci.[7,8] Azithromycin alone and in combination is prescribed for the treatment of COVID-19.[9]

The analysis of Azithromycin by high performance liquid chromatography (HPLC)[10-15] is widely known and present in pharmacopeial compendiums, other methods like voltammetry assay,[16] amperometry,[17] spectrofluorometer,[18] capillary electrophoresis,[19,20] HPLC-MS/MS,[21] HPLC-MS-MS,[22] and high-performance thin-layer chromatography (HPTLC) [23-27] are present in literature.

The current need and focus on developing an analytical method to analyze Azithromycin in simple and precise way.

Sultana *et al*.[28] studied the degradation aspects of Azithromycin in an acidic and basic medium by varying the concentration, temperature and time. His choice of reagents was $H_2SO_4$, $H_3PO_4$, HCl, $HNO_3$, NaOH, KOH and $NH_3$. Though the developed process was lengthy, it was found that concentrated $H_2SO_4$ is an ideal candidate reagent for the estimation of Azithromycin.[28]

The method followed by Kumar *et al*.[29] is involved heating with $H_2SO_4$ to develop colour. The procedure involved preparation of stock solution in 0.01 M of phosphate with pH 7.5 to get a concentration of 1 mg/mL, which practically was found to give suspension rather than solution.[29]

Bhimani *et al*.,[30] proposed using 0.1 N HCl for solubilization and performed estimation at lambda max 208 nm. Measuring absorbance in near range of 200 nm may pose problems due to cut off wavelength of solvents.[30]

The UV analysis of the drug-using charge transfer complex[31-35] is a growing trend. Involves the use of charge-transfer complex or electron-donor acceptor complex in an association with one or more molecules at different sites of a molecule or molecules, and contrary to the other part, the extracting procedure also makes it tedious and time-consuming. The UV analysis using oxidation and reduction principle[36-39] involves reagents. Their standardization and stability are questionable during method development, which could create an erroneous result.

Though the use of $H_2SO_4$ for estimation of azithromycin is mentioned in the literature, the processes were lengthy. We developed the process to carry out analysis at room temperature and the process was successfully applied to dissolution studies.

## MATERIAL

- Instrument: UV-visible double beam spectrophotometer with matched quartz cells (1-cm)

*MODEL:* THERMO EVOLUTION SCIENTIFIC UV-550
MAKE Thermo Scientific, 81 Wyman Street Waltham, Massachusetts, US.

- Chemical Reagents: Concentrated $H_2SO_4$ (Merck analytical grade), Azithromycin Dihydrate obtained as a gift sample from Ajanta pharmaceuticals and Distilled water obtained from in house plant.

## METHOD

*Preparation of Standard Stock Solution*

Azithromycin dihydrate equivalent to 100 mg of azithromycin was transferred to a standard 100 mL volumetric flask. The volume was adjusted to 100 mL using 0.1M $H_2S0_4$ to get a 1-mg/mL. Further dilutions were done in 5 mL of standard volumetric flask. 2.5 mL of (18M) Conc $H_2SO_4$ was added. Required stock solution was added to get serial dilutions of concentration ranges 20, 30, 40, 50, and 60 µg/mL. The volume was adjusted using single-pass distilled water. The prepared dilutions were kept at room temperature 24ºC for 1-hour, a yellow solution was obtained, and absorbance was measured at a fixed wavelength of 480 nm using a UV-visible spectrophotometer.

*Preparation of Sample Solution*

Azithromycin mucoadhesive buccal tablets were finely triturated. Triturate equivalent to 100 mg of azithromycinn was weighed accurately and transferred to 100 mL standard volumetric flask, and the volume was adjusted to 100 mL using 0.1M $H_2S0_4$ to get a concentration of 1 mg/mL. The required solution was filtered through a 0.2 µm PVDF filter, and this filtrate was used for analysis.

## DISSOLUTION STUDIES

Dissolution of mucoadhesive buccal tablets and marketed tablets was done USP type 2 apparatus with dissolution media consisting of 250 mL of phosphate buffer pH 6.0 rotating at 50 rpm at 37ºC. Samples were withdrawn at 1, 2, 4, 6, 8 hours and replaced with fresh buffer media to maintain the sink conditions. These samples were filtered through PVDF HPLC syringe filter before analysis.

## VALIDATION OF ANALYTICAL METHOD

### Selectivity and Specificity

The placebo formulation was prepared and assessed similar to sample compared with drug-containing formulation showed no interference

### Linearity and Range

Standard solutions of AZI with a 20 to 60 µg/mL concentration range were prepared from a 1000 µg/mL stock solution, and absorbance was measured. Six replicates were carried out. The calibration curve was constructed by plotting the concentration level of drug versus absorbance

### Precision

At three levels, 20, 40, 60 six replicates for repeatability and three replicates for intraday for three-time points each and six replicates for inter-day studies for three different days were carried out to determine intermediate precision and reproducibility different, analyst using 3 concentration levels with six replicates done for this method.

### Accuracy

Accuracy studies were carried out with the standard solution of 20, 40 and 60 ppm. Six replicates were carried out % recovery of the drug and %RSD, at different levels were estimated.

### Limit of Detection (LoD), and Limit of Quantitation (LoQ)

The LoD and LoQ was calculated based on a standard deviation of the y-intercept of the calibration curve and slope.

The LoD may be expressed as:

$$LoD = 3.3 \times \sigma/S$$

The LoQ may be expressed as:

$$LoQ = 10 \times \sigma/S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The estimation of slope (S) was done from the data obtained from the calibration curve of the analyte. The estimate of σ was be carried out using the standard deviation of the y-intercept of the calibration curve.

### Robustness

The absorbance of the standard solution was measured at 479 and 481 nm. The percent recovery was calculated. The %RSD for the wavelength of 479. 480. 481 nm was calculated

## RESULTS AND DISCUSSION

### Selectivity and Specificity

The spectra analysis shows that the Azithromycin mucoadhesive buccal tablets did not interfere with the developed method Figure 2. The spectrum showed that the placebo did not have absorbance in the wavelength used in this method.

### Linearity and Range

The analytical curves, obtained for six replicates on three consecutive days (n = 6) by plotting the mean of absorbance at 480 nm against the concentration, were found to be linear in the range of 20 to 60 µg/mL and yielded a correlation coefficient of 0.9997 (Figures 3 and 4, Table 1 and 2).

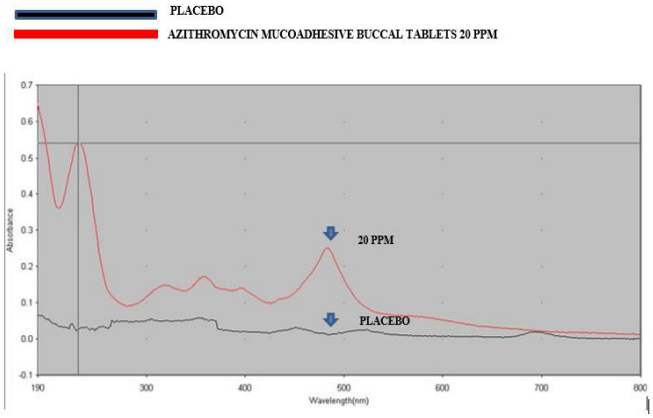


**Figure 2:** Placebo and formulation spectrum

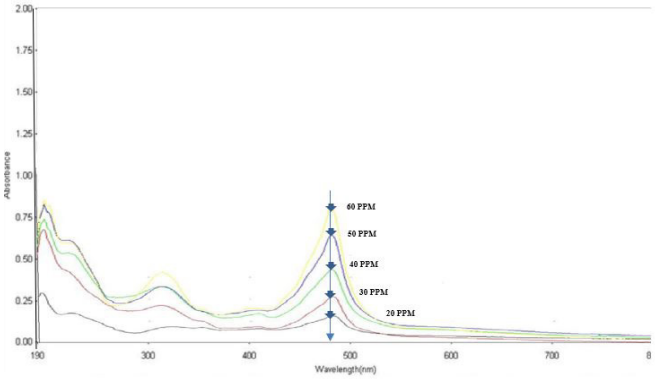


**Figure 3:** UV spectra of azithromycin dihydrate with different concentration range

### Precision

The precision, evaluated as the repeatability of the analytical method, was studied by calculating the %RSD for the six replicates of 20, 40, 60 µg/mL working standard solution performed on the same day and under the same experimental conditions. The obtained %RSD was less than 2.0%. The intermediate precision was assessed by analyzing it on 3 different days (inter-day precision) six replicates and (intraday) three-time points each time points Morning, afternoon and evening 3 replicates for each time point and reproducibility analyzing the samples with different lab (inter-laboratory precision). The %RSD. values were less than 2.0%, confirming that the method is sufficiently precise (Table 2).

### Accuracy

The accuracy of the proposed method was assessed by determining the average recoveries of samples using the standard addition method. Mean recovery was 99.25%, and %RSD was less than 2%. The results were under fixed limits of 98.0 to 102%, indicating the suitability of the developed method. The accuracy value of the current method was found excellent.

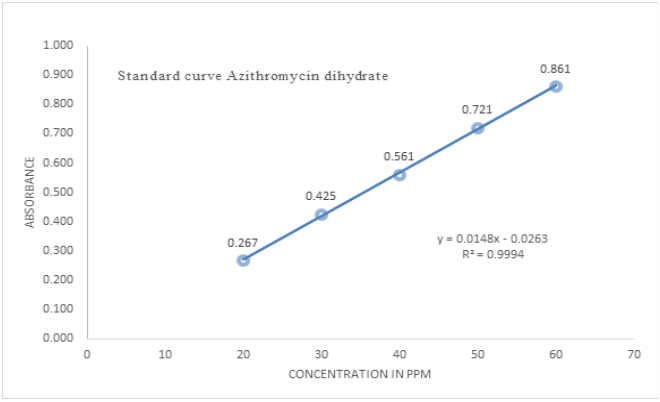


**Figure 4:** Linearity curve of azithromycin dihydrate

**Table 1:** Results of linearity and range

| *Concentration in PPM* | *Average* | *Standard Deviation* | *%RSD* |
|---|---|---|---|
| 20 | 0.266 | 0.968 | 0.968 |
| 30 | 0.425 | 1.040 | 1.040 |
| 40 | 0.56 | 0.990 | 0.990 |
| 50 | 0.72 | 0.753 | 0.753 |
| 60 | 0.86 | 0.962 | 0.962 |

**Table 2:** Results of the linear curve of azithromycin dihydrate

| *Parameter* | *Result* |
|---|---|
| Linearity Range | 20-60 µg/mL |
| Slope | 0.0148 |
| Intercept | -0.0263 |
| Correlation Coefficient | 0.9994 |

### Limit of Detection (LoD) and Limit of Quantitation (LoQ)

For this analytical method, when calculated as mentioned above, LoD and LoQ values were found to be 1.936 and 5.868 µg/mL, respectively.

### Robustness

The robustness was found reliable, as determined by %RSD (< 2%). The percent recovery was calculated using the standard equation obtained, and the 100 % content considered the amount

**Table 3:** Repeatability - inter day precision results

| | | | *conc ppm* | *1* | *2* | *3* | *4* | *5* | *6* | *%RSD* |
|---|---|---|---|---|---|---|---|---|---|---|
| Inter day | Repeatability | | 20 | 0.263 | 0.267 | 0.262 | 0.264 | 0.272 | 0.265 | 1.363 |
| | | | 40 | 0.565 | 0.55 | 0.558 | 0.555 | 0.571 | 0.56 | 1.324 |
| | | | 60 | 0.89 | 0.88 | 0.85 | 0.883 | 0.86 | 0.87 | 1.731 |
| | Intermediate precision | DAY 1 | 20 | 0.266 | 0.265 | 0.274 | 0.271 | 0.268 | 0.262 | 1.607 |
| | | | 40 | 0.55 | 0.548 | 0.564 | 0.565 | 0.54 | 0.56 | 1.813 |
| | | | 60 | 0.859 | 0.871 | 0.863 | 0.88 | 0.863 | 0.87 | 0.874 |
| | | DAY2 | 20 | 0.272 | 0.267 | 0.265 | 0.262 | 0.265 | 0.264 | 1.289 |
| | | | 40 | 0.562 | 0.568 | 0.565 | 0.557 | 0.564 | 0.553 | 0.984 |
| | | | 60 | 0.883 | 0.856 | 0.879 | 0.861 | 0.87 | 0.85 | 1.502 |
| | | DAY 3 | 20 | 0.263 | 0.264 | 0.262 | 0.264 | 0.264 | 0.272 | 1.367 |
| | | | 40 | 0.558 | 0.564 | 0.567 | 0.562 | 0.564 | 0.55 | 1.080 |
| | | | 60 | 0.85 | 0.87 | 0.863 | 0.882 | 0.858 | 0.87 | 1.283 |

**Table 4:** Repeatability - Intra day precision results

| | | | *conc ppm* | *1* | *2* | *3* | *1* | *2* | *3* | *1* | *2* | *3* | *%RSD* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Time point 1* | | | *Time point 2* | | | *Time point 3* | | | |
| Intraday | Intermediate precision | Day 1 | 20 | 0.265 | 0.264 | 0.258 | 0.271 | 0.266 | 0.264 | 0.267 | 0.266 | 0.268 | 1.333 |
| | | | 40 | 0.564 | 0.563 | 0.55 | 0.549 | 0.563 | 0.563 | 0.558 | 0.559 | 0.564 | 1.057 |
| | | | 60 | 0.85 | 0.874 | 0.88 | 0.845 | 0.88 | 0.87 | 0.867 | 0.86 | 0.874 | 1.452 |

**Table 5:** Reproducibility precision results

| | | *Conc PPM* | *1* | *2* | *3* | *4* | *5* | *6* | *%RSD* |
|---|---|---|---|---|---|---|---|---|---|
| Reproducibility | Lab a | 20 | 0.262 | 0.266 | 0.265 | 0.264 | 0.264 | 0.265 | 0.517 |
| | | 40 | 0.557 | 0.564 | 0.563 | 0.566 | 0.564 | 0.563 | 0.544 |
| | | 60 | 0.855 | 0.862 | 0.865 | 0.874 | 0.87 | 0.89 | 1.387 |
| | Lab b | 20 | 0.269 | 0.269 | 0.261 | 0.264 | 0.268 | 0.266 | 1.198 |
| | | 40 | 0.558 | 0.57 | 0.553 | 0.56 | 0.563 | 0.559 | 1.014 |
| | | 60 | 0.862 | 0.853 | 0.869 | 0.86 | 0.87 | 0.873 | 0.868 |

**Table 6:** Accuracy results

| *Amount of drug added* | *Mean absorbance found (N=6)* | *Percent recovery* | *Standard deviation of % recovery* | *%RSD* | *Mean recovery* |
|---|---|---|---|---|---|
| 20 | 0.264 | 98.074 | 0.0041 | 1.733 | 99.25% |
| 40 | 0.563 | 99.544 | 0.0085 | 1.563 | |
| 60 | 0.863 | 100.146 | 0.014 | 1.619 | |

Table 7: Robustness results

| *Sr.No.* | *Amount present PPM* | *%Recover at 479* | *% Recovery at 480* | *% Recovery at 481* | *%RSD* |
|---|---|---|---|---|---|
| 1 | 40 | 98.31 | 99.22 | 100.063 | 1.35 |
| 2 | 40 | 97.03 | 100.21 | 102.13 | |
| 3 | 40 | 96.87 | 98.22 | 99.58 | |
| 4 | 40 | 97.67 | 98.66 | 98.78 | |
| 5 | 40 | 98.94 | 99.23 | 100.06 | |
| 6 | 40 | 97.35 | 99.21 | 100.54 | |

**Table 8:** Results for Mucoadhesive buccal tablets of Azithromycin Dihydrate

| *Sr No.* | *Content In MG* | *% Recovery* | *Standard deviation* | *%RSD* |
|---|---|---|---|---|
| 1 | 99.17 | 99.17 | | |
| 2 | 100.76 | 100.76 | | |
| 3 | 99.8 | 99.8 | | |
| 4 | 102.35 | 102.35 | 1.34 | 1.339 |
| 5 | 98.53 | 98.53 | | |
| 6 | 100.44 | 100.44 | | |
| | Average (N=6) | 100.175 | | |

**Table 9:** Results for marketed tablets azipro of azithromycin dihydrate

| *Sr. No* | *Content in MG* | *% Recovery* | *Standard Deviation* | *%RSD* |
|---|---|---|---|---|
| 1 | 100.23 | 100.23 | | |
| 2 | 98.5 | 98.5 | | |
| 3 | 102.1 | 102.1 | | |
| 4 | 99.8 | 99.8 | 1.036 | 1.032 |
| 5 | 100.34 | 100.34 | | |
| 6 | 101.2 | 101.2 | | |
| | AVERAGE (N=6) | 100.36 | | |

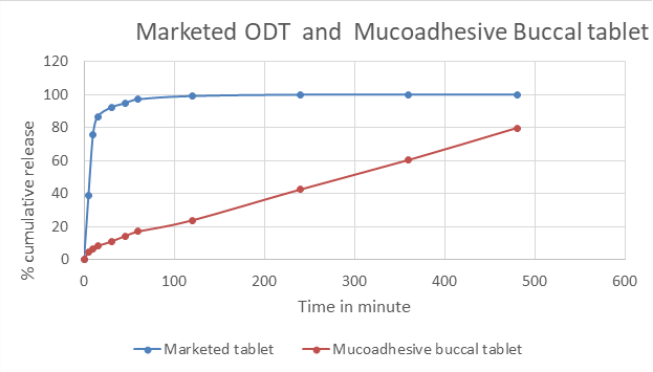


**Figure 5:** Dissolution of marketed and mucoadhesive buccal tablet

added to the volumetric flask. It was observed that the constancy of the absorbance with deliberative changes in the experimental parameter of wavelength resulted in a %RSD less than 2%. The minor changes that occurred during the analysis did not majorly affect the absorbance of the samples (Tables 3–7).

## ASSAY OF FORMULATION

This analytical method was successfully applied to Azithromycin mucoadhesive buccal tablets. 6 The samples were analyzed for the drug content (100mg) in the formulation, and they were found to be within limits of 92.5–110% as per IP (Indian pharmacopeia) (Tables 8–10).

The same method was applied to the marketed tablets of Azipro 100mg DT 3s. The drug content and results found were in accordance with IP

## DISSOLUTION STUDIES

Dissolution of mucoadhesive buccal tablet was evaluated, which showed 80 % cumulative drug release at 8 hours within in-house limits of ± 2.5 %. The Dissolution of marketed tablet Azipro 100mg DT 3s was evaluated as per IP having % cumulative drug release was 94.3 % at 45 minutes. This indicates that the developed method developed can be successfully applied for the formulation.

## SUMMARY OF OPTICAL AND VALIDATION PARAMETERS

**Table 10:** Summary table for optical and validation parameters

| *Parameter* | *Result* |
|---|---|
| Lambda max (λmax) nm | 480 |
| Linearity range | 20-60µg/mL |
| Regression equation | Y = 0.0148X - 0.0266 |
| Correlation coefficient ($R^2$) | 0.9994 |
| Slope | 0.0148 |
| Intercept | -0.0266 |
| Precison | 0.868 – 1.813 %RSD |
| Accuracy | 99.25 % RECOVERY |
| LoD | 1.936 µg/mL |
| LoQ | 5.868 µg/mL |
| Robustness | 1.35 %RSD |

## CONCLUSION

The analytical method developed and validated for quantitative estimation of azithromycin formulation, which was simple, less time-consuming, and required minimum sample treatment, enhancing high analytical productivity. The validation parameters were in line and within limits. This method is useful for quality control parameters for azithromycin formulation and is simple to previous methods.

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