RESEARCH ARTICLE

Spectrophotometric Determination of Azithromycin using Oxidative Coupling Reaction

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ABSTRACT

For determining the azithromycin (AZT) in medicinal and pure formulas, a simple spectrophotometric technique was developed. An approach suggested is dependent on an AZT's oxidative coupling reaction by sodium period (SPI) and 4-amino antipyrine (AAP) producing a pink colored compound with optimum absorption of 480 nm. Different experimental parameters are extensively researched and mastered, which affects the stability of a colored product formed, then developed. The law of the Beer is obeyed over its concentration range 3 to 44 ppm, whereas the limit of detection and quantification is 0.1908 and 0.5726 ppm, respectively, for a connection factor (r) = 0.9998. Also calculated are the molar absorption of 8.23×10^3 L/mol.cm, and the sensitivity index for Sandell is 7×10^{-5} mg/cm^2. A method's accuracy and precision are tested by also determining a relative standard deviation (RSD) < 0.645 percent, and 100.189 percent average recovery. Practically possible external interferences about a calculation for AZT are checked in drug tablets. The results demonstrated that the procedure for determining AZT was successful in its application in pharmaceutical preparations. Comparing the literature survey that shows good sensitivity and selectivity, the reliability of the proposed method is chalked in.

Keywords: 4-Aminoantipyrine, Azithromycin, Oxidative coupling, Spectrophotometric.

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INTRODUCTION

Azithromycin (AZT),[1] an antibiotic macrolide that works for gram-negative and gram-positive bacteria (Figure 1).[2,3] It is doing its antimicrobial action through inhibiting the synthesis of proteins, reversibly binding the bacteria's 50S ribosomal subunit to the "P" site.[4,5] It is used in many adults and pediatrics,[6,7] respiratory tract infections,[8-10] skin, soft tissue infections, medium otitis,[8,11,12] sinusitis, pharyngitis, cystic fibrosis, severe bronchitis, GIT infections,[13,14] tonsils infection,[15] anti-inflammatory in chronic obstructive pulmonary disease patient,[16] in *Plasmodium* sickle-cell malaria and other anti-malarial medication,[17] and fever from typhoid.[18,19] AZT is an erythromycin derivative; however, this is structurally different from erythromycin in the lactone ring and related semi-synthetic erythromycin derivatives incorporate methyl-substituted nitrogen atom.[20] This indicates a broader range of activities, with acid, has better stabilization, by oral, there is better bio-availability, and greater kinetic activity from erythromycin.[21] The remarkable kinetic characteristics include broad tissue distribution with highly concentrated drugs inside the cells. The most innovative feature of the oral 3-day diet is effectiveness and safety.[22] The normal AZT dosage is between 100 to 500 mg per day. A study survey shows that several techniques to evaluate AZT are used in pharmaceutical applications that include HPLC,[23-30] thin-layer chromatography,[31] micellar liquid chromatography,[32,33] voltammetry,[34,35] Fourier transform infrared (FTIR),[36] flow-injection chemiluminescence,[37] flow-injection analysis and amperometric detection,[38,39] fluorescence,[40] spectrofluorometry,[41] and UV-vis spectrophotometry.[42-50]

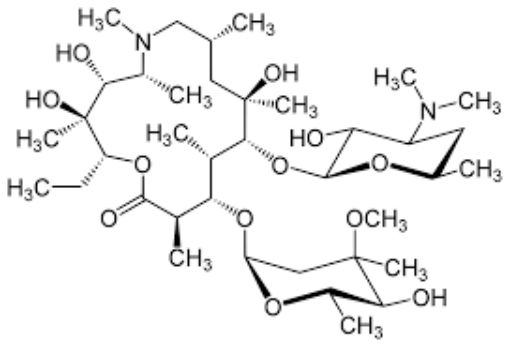

Figure 1: Structure of AZT

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Microbiological approaches ensure inside living organism studies for evaluating the effectiveness of antibiotics toward sensitive organisms. Several of the stated spectrophotometric approaches were correlated to disadvantages, including reduced test procedure simplicity, e.g., tedious separation steps in methods based on ion-pair formation, using some solvents that are harmful to human health. The purpose of this report was to develop a particularly good, easy, and spectral method, for identifying large quantities of drug formulations on the market, based on interaction with functional groups.[51,52]

MATERIALS AND METHODS

Apparatus

A double beam Shimadzu 160A ultraviolet/visible spectrophotometer, many tests (Jenway, 7305-UK) ultraviolet/visible are performed; spectrophotometer fitted to 1-cm pathway diameter quartz cell. The pH meter (Philips PW 9421), water bath (LA Bacon LWB-104), digital analytical balance (Mettler Toledo AB 204-S), and magnetic stirrer hotplate (Gallenkamp-400), are used through this investigation.

Solutions and Reagents

The analytical grade is used for all chemicals and reagents. Samarra Pharmaceutical Factory (SDI) in Iraq supplies AZT. Dis.W, for preparations, have been used.

AZT Solution (1,000 mg/L)

A 0.1-gram of AZT is dissolved in 1-mL 6M HCl, and then into a volumetric flask made up to 100 mL of Dis.W water. Works solutions with 250 mg/L were made from diluting storage solutions, then packed into a refrigerator in a dark volumetric flask.

AAP Solution (0.1M)

2.0324 grams of 4-amino antipyrine is dissolved in Dis.W, and diluted in a volumetric flask to 100 mL.

SPI Solution (0.1M)

2.1389 grams of sodium periodate is dissolved in Dis.W, and finished to 100 mL for Dis.W to this preparation.

Hydrochloric Acid Solution (6M)

51 mL of concentrated HCl solution (11.8M, 37%) was diluted to 0.1-liter volumetric flask with DW.

Sample of Tablet's Solution containing AZT (250 ppm)

From 2 different companies, 20 tablets are weighed (each tablet contains 500, 250 mg AZT), and granulated to fine particles, then apportion equivalent to 250 μg mL^{-1} of AZT is weighed into 100 mL volumetric flask, then the solution filtered by filter paper (589/4 SandS Rundfilter), then completed into the mark for each type separately.

RESULTS AND DISCUSSION

Preliminary Experiments

Added 1-mL (0.1M) of SPI to 25 mL, then added 2 mL standard AZT (250 ppm), followed by adding 1-mL (0.1M) of AAP in the acid medium, using 1-mL (6M) of HCl, then diluted with distilled water to the level of volumetric flask. Except for AZT, a blank was prepared similarly. The colored complex (pink color) absorption spectrum against a blank solution indicates full absorption at 480 nm.

Selection of Acid and its Concentration

This study was conducted using different types of strong and weak acids with a concentration of 6M to see the effect of different acids and choose the best. It found that hydrochloric acid gives the highest absorbance. 0.1 to 3 mL of HCl (6M) has been added for a number of volumetric flasks (25 mL) that contain 2 mL of AZT solution (250 ppm) and 1 mL SPI solution (0.1M), then waited for 2 minutes, and added 1-mL of AAP solution (0.1M), and completed to mark with Dis.W. It was found that the best amount for hydrochloric acid is 1.2 mL, the highest absorbance, then the pH of the final solution found to be 0.7.

Selection of Type of Oxidizing Agent and its Concentration

The effects of various oxidizing agent types (1-mL, 0.1M) are tested. Results indicate that SPI gives the best maximum absorption, so the impact of its volume, within the limits 0.3 to 4 mL (0.1M) on the reaction sensitivity, was studied. It is found that the optimum absorbance is given by 2 mL of SPI (0.1M).

Selection of Type of Coupling Reagent and its Concentration

Specific coupling reagents are evaluated based on the highest value of maximum wavelength. AAP was found to be the best reagent for coupling because it provides the maximum concentration possible of λ_{max} = 107 nm (λ_{max} of sample agents blank = 480 nm, and agents water = 313 nm).

The effect of AAP solution (0.1M) to a constant amount of AZT (2 mL, 250 ppm) within the range of 0.3 to 3 mL has been investigated. It was found that the color's maximal strength is at 1.5 mL, as the additional change in quantity resulted in an almost stable absorption.

Additions Arrangement

An influence for additions arrangement at the method's sensibility was investigated. Results obtained that the addition order [drug sample (AZT) + HCl + SPI + AAP] appears to be of the good sensibility with more intensive coloring than the other probabilities, then that arrangement is chosen to all following experiments.

Oxidation Time Effect and Temperature Effect

The oxidation of AZT by SPI in acidic medium (HCl) is checked over various periods (1–30 minutes), and then the AAP is used as a coupling reagent. It has seen the best complexing develop after 7 minutes. For the subsequent tests, therefore, 7 minutes will be chosen, because there is strong stability in the absorbance at this time. Additionally, the effect on temperature has been examined between 4 and 55ºC. This showed that the maximum absorbance within the region of 20 to 30ºC can be reported, so for subsequent experiments, considered 25ºC (room temperature).

Color Product Stability

The formed product stability was tested over different times (0–65 minutes). The absorbance measurement is found to be stable up to 55 minutes, which is adequate for an appropriate determination.

Effect of the Solvents

A number of organic solvents (acetone, ethyl acetate, diethyl ether, acrinitral, methanol, and ethanol) have been taken to study its impact instead of water. It is found that using water as solvent gives the highest absorbance.

Procedure Suggested

To a 25 mL calibrated flask containing 2 mL at 250 ppm an AZT's standard solution of eq 20 ppm, then added 1.2 mL of HCl (6M) and 2 mL SPI (0.1M), then added 1.5 mL AAP (0.1M) after 7 minutes, and filled the mark with Dis.W. In the absence of AZT, exactly in the same way, a solution of blank was prepared. The absorbance to 480 nm is measured against a blank reagent. An unknown amount was derived from the calibration diagram or was calculated using Beer′s law from the regression equation.

Diagram Calibration and Statistics

A test diagram can be obtained after using various volumes of AZT (0.05–6 mL, 250 ppm) under the optimal experimental conditions, and by applying the suggested method (0.5–60 ppm). Figure 2 shows a straight line of the calibration diagram at the amount limit 3 to 44 ppm AZT, and a correlation factor 0.9998 and 0.1908 ppm detection limit. It is found that the molar absorptivity and the Sandell index are 8.23×10^3 L/mol.cm and 0.07 μg/cm^2, respectively.

Statistical Analysis

In this test, the absorbance for various amounts (5, 23, and 42 ppm) of AZT is calculated for (n = 10) ten repetition times. Measurement has been illustrated in Table 1, which shows the best precision, as well as, the best accuracy as recovery percent, then RSD reaches 100.189 and 0.645 percent, respectively.

Stoichiometric Relation between AAP and Oxidized AZT

The stoichiometric relationship between oxidized AZT and AAP was studied by using the molar ratio method. The results are shown in Figure 3, which shows that the reaction between the reagent and the oxidized AZT is 1:1. From these results, we were able to calculate the stability of the resulting complex and found equal 7.6×10^7 L/mol; this result shows high stability for the complex.

Interferences Effect

To improve the effectiveness and specificity to a suggested procedure for determining AZT, influence a certain additives chemicals (starch, maltose, glucose, and fructose) that are normally existing in the drug compositions of pharmaceutical preparation, has been researched. The results found that absorbance's difference of AZT's drug, and AZT and interferences together = ±5 percent for recoveries, a substance is considered not to interfere. Table 2 illustrated the recovery

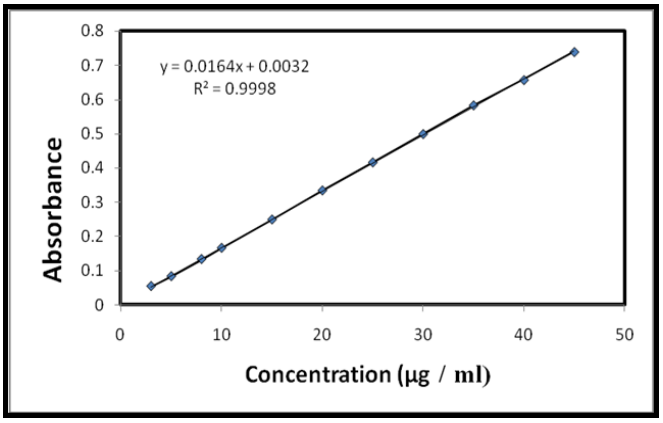

Figure 2: Diagram calibration for calculation of AZT

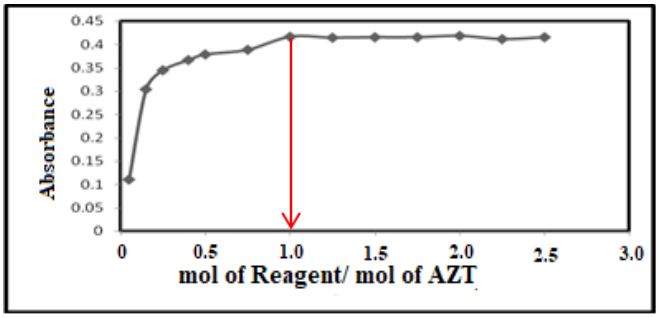

Figure 3: Molar ratio for oxidative drug AZT coupled with AAP

Table 1: Accuracy and precision results for determining AZT

Concentration of AZT (ppm)	*Rec (%)**	*Med Rec (%)*	*RSD %*
5	100.36		0.645
23	100.541	100.189	0.598
42	99.668		0.328

*Average of ten determinations (n = 10)

Table 2: Interferences effect

	Rec (%) of 20 ppm of AZT per ppm Added the additives		
Additives	*60*	*120*	*240*
Glucose	99.69	101.47	99.75
Fructose	100.65	100.65	100.47
Maltose	100.12	100.06	99.69
Starch	99.75	100.36	100.48

Table 3: Simple process for capsules determination in drug formulations

Drug formulations capsules	*AZT tested (ppm)*	*AZT measured (ppm)*	*Rec%*	*RSD%*
ZIMAX 250 **mg** tablet SPIMACO	12	12.031	100.258	0.087
	27	26.85	99.444	0.186
	38	37.949	99.865	0.142
ZEROX 500 **mg** tablet HIKMA	12	11.95	99.583	0.14
	27	26.927	99.729	0.078
	38	37.913	99.771	0.076

Table 4: Comparison of suggested method with literatures

Analytical parameter	*[47]*	*[48]*	*Suggested method*
Reagents	Acetyl acetone-ammonium acetate	1,10-phenanthroline	4-aminoantipyrine
Range of Beer's law **(µg/**mL)	10–75	2.5–15	3–44
ε (L/mol.cm)	8,718.4	9,600	8,230
Sandel index **(µ**g/cm^2)	0.086	0.0811	0.07
pH	-	-	0.7
Temperature ºC	37	45	20–30
λ_{max} (nm)	412	490	480
Reco. (%)	98.3–101.7	-	100.189
RSD (%)	0.118	0.97	0.328–0.645
Solvent	Oxalic acid	Water	Water
Stability constant (L/mol)	-	-	7.6×10^7
Color of drug	Yellow	Red	Pink
Nature of drug	-	-	1:1
Pharmaceutical preparation	Capsules, tablets	Tablets	Tablets

percentage by 3 to 12 folds greater than the amount of AZT, after having added the additives.

Application

Different volumes (1.2, 2.7, and 3.8 mL) of a solution for pharmaceutical formulations (250 µg/mL), as shown in Table 3, turned to 25 mL volumetric flasks, while the volumes completed with water and the concentrations became 12, 27, and 38 µg/mL. The optimal conditions for measuring the absorption were applied at 480 nm, each measurement is replicated five times (n = 5). The recovery and RSD are estimated to approximate the precision and accuracy of the suggested process.

Comparison of Suggested Method with Literatures

Table 4 shows the comparison of some of the analytical variables for those of other spectrophotometric methods with the present method. Complexity was found in the proposed method in water as a solvent and at room temperature, while other methods occur at elevated temperatures, and in solvents other than water.

CONCLUSION

A procedure is found that is simple, fast, responsive, cheap, and exact to determine AZT in medicinal and pure formulas. The process did not request the removal of excipient, pretreatment of drugs samples, solvent separation, and also, costly use of chemicals.

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