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SIMPLEX OPTIMIZATION FOR THE SPECTROPHOTOMETRIC DETERMINATION OF AZITHROMYCIN DRUG VIA ION-PAIR FORMATION

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Abstract: A spectrophotometric determination of azithromycin was optimized using the simplex model. The approach proved to be accurate and sensitive. The analyte reacted with bromothymol blue (BTB) to form a colored ion pair, which was extracted in chloroform in a buffer medium of pH 4 potassium phthalate. The extracted colored product was assayed at 415 nm, exhibiting a linear quantification range of 1-20 µg/mL. The LOD was 0.671 µg/mL, with a correlation coefficient of 0.9998 and an RSD% of 0.96±0.2. The molar absorptivity was 20253.5 L/mol·cm. The excipients did not interfere with the proposed method for assaying azithromycin in curative formulations.

Keywords: *Azithromycin, spectrophotometric, simplex, ion-pair.*

1. Introduction

A Croatian research team made the initial discovery of azithromycin, also known as "9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin" (Deming et al., 1988). This medication is effective against diverse types of bacteria, including chlamydia, legionella, and mycobacteria (Dinos, 2017). It functions as an inhibitor of bacterial protein synthesis. Azithromycin has been approved by the Food and Drug Administration (FDA) for the treatment of pneumonia (Dekate et al., 2011). It is also approved for treating several upper respiratory infections, including acute obstructive pulmonary disease flare-ups and otitis media (MJ, 2014). Its chemical composition is represented in Figure 1.

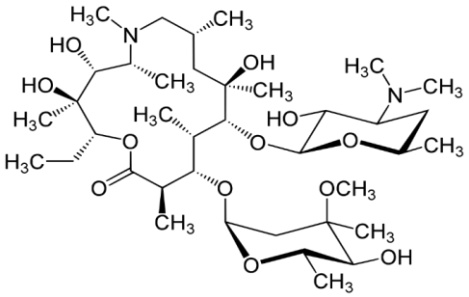

Figure 1. Molecular configuration of azithromycin.

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The determination of azithromycin has been achieved through various spectrophotometric techniques (Abdullah et al., 2014; Chiluka & Raut, 2022; Devi, 2011; Doan et al., 2023; El-Yazbi et al., 2020; Gulhane et al., 2021; Ibrahim et al., 2017; Jayanna et al., 2012; Rufino et al., 2008; Sayanna et al., 2019; Suhagia et al., 2006; Walash et al., 2007). Spendley (1962) introduced the use of simplex optimization, which was later refined by Nelder and Aberg (Åberg & Gustavsson, 1982; Nelder, 1965). This method has various applications in analytical chemistry (Michałowska-Kaczmarczyk & Michałowski, 2014; Momenbeik et al., 2005; Pulgarín et al., 2002; Tinoi et al., 2005) and is a geometric form where (n) denotes the number of variables. It relies on a statistical strategy search to determine the maximum or minimum responses by eliminating the worst point and substituting a new point.

The present work describes a modified simplex technique for spectral quantifying azithromycin (as dihydrate) using BTB as a chromogenic reagent. The optimization of chemical-dependent factors was also studied using a computer program.

2. Materials and Methods

Apparatus

Shimadzu-1800 UV-vis spectrophotometer, pH meter (DW942) Philips, Sartorius balance (210S).

Experimental

SDI provided standard powders of azithromycin (as dihydrate), The State Company for Drug Industries and Medical Appliances. A 0.1g of BTB was dissolved in 5 mL of methyl alcohol, and the remaining 95 mL were filled with distilled water to create a solution containing 0.10% (w/v) of BTB. A 0.10 M hydrochloric

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acid solution was prepared by combining 0.850 mL of hydrochloric acid (with a specific gravity of 1.18 and concentration of 37%) with 50 mL of distilled water.

A 0.10 M potassium hydroxide solution was prepared by dissolving 0.560 g of KOH in 100 mL of distilled water. A 0.20 M phthalate buffer was also created by dissolving 4.08 g of potassium hydrogen phthalate in 100 mL of distilled water.

Standard Solution

By dissolving 50 mg analyte in 5 mL methanol then diluting it to 100 mL distilled water, a standard solution of 500 µg/mL azithromycin (dehydrated) was created.

General Procedure

In total, 1 mL aliquots of the medication, 0.5 mL of pH 4.0 phthalate buffer, and 0.25 mL of 0.1% BTB were added to a 50 mL separating funnel. After shaking the separatory funnels for five minutes with five milliliters of chloroform, the absorbance of the colored chloroform phase was measured at 415 nm against a blank.

Assaying Azithromycin in Medications

Ten tablets and ten capsules were separately ground into fine powder. Then, 500 mg of the powdered tablets and 250 mg of the powdered capsules were each dissolved in 10 mL of methyl alcohol in separate volumetric flasks. The volumes were then made up to 100 mL with distilled water and filtered.

3. Results and Discussion

Ion-pair extractive spectrophotometry can determine many pharmaceutical formulations (Basavaiah et al., 2007; Milano & Cardoso, 2005; Siddappa et al., 2008). Azithromycin reacts in an acidic pH with Bromothymol Blue to produce a yellowish product that is soluble in chloroform. The electronic transition n→π* occurs between the nitrogen atom in the azacyclopentadecan ring and the sulfur atom in the BTB reagent, resulting in an ion-pair complex. This transition shifts the absorption from the ultraviolet region for azithromycin to the visible region, specifically at 415 nm (Figure 2). This shift permits optimal analytical conditions for the examination of the drug in its dosage form.

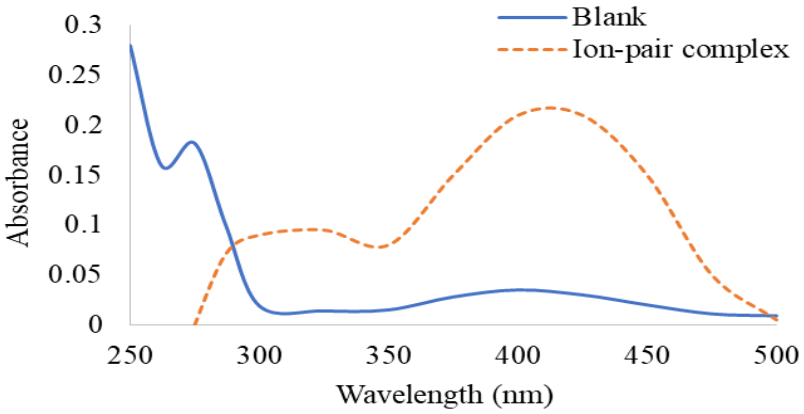

Figure 2. Absorption of the ion-pair complex of azithromycin (10 µg/mL) vs blank.

Optimization by Simplex Method

The simplex approach was employed to optimize the pH, reagent quantities, and mixing duration (Table 1). Initially, one of the four major parameters was selected based on its impact on the absorption signal of the colored complex. The absorbance values for these four experiments were measured, and the output of the simplex program is shown in Table 2. The least significant point was replaced with a new one, and the program continued to run. A measured signal was fed back into the computer, and the process was repeated until optimal conditions were achieved (Table 3). The variable setting was then used as a reference point in subsequent experiments.

Table 1. Boundary variables of the study.

Variable	Data Range
pH	3-6
Reagent Vol. (mL)	0. 1-0.25
Shaking time (min.)	1-5

Table 2. Simplex program of the first four experiments.

#	pH	BTB Vol. (mL)	Shaking Time (min.)	Absorbance
I	4.00	0.10	1.0	0.163
II	5.50	0.20	2.0	0.230
III	6.00	0.15	4.0	0.024
IV	3.00	0.25	3.0	0.225

Effect of pH

It was observed that pH 4.0 yielded the highest color intensity and stable absorbance values (Table 3). Solutions with higher or lower pH values than this optimal value exhibited decreased absorbance due to forming a new absorbing species. Given that azithromycin's pKa is 8.85 (McFarland et al., 1997), it is evident that at a pH lower than this value, the antibiotic is likely to be protonated, and at a higher pH, it will be deprotonated (Sayle, 2000). This supports the hypothesis that an ion pair would form. Consequently, all subsequent experiments were conducted at pH 4.0.

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Table 3. Experimental Simplex program.

pH	BTB Vol. (mL)	Shaking Time (min.)	Absorbance
6.0	0.25	5	0.238
6.0	0.20	5	0.202
4.5	0.25	4	0.245
5.0	0.25	5	0.234
4.0	0.25	5	0.256
3.0	0.25	5	0.212
5.0	0.25	5	0.234
5.0	0.25	5	0.211
4.5	0.25	4	0.245
3.5	0.25	5	0.240
4.5	0.25	4	0.245
3.5	0.25	5	0.240
4.5	0.25	4	0.245
3.5	0.25	5	0.240
4.5	0.25	4	0.245
4.5	0.25	4	0.245

Calibration Graph

Under Under optimal conditions, a linear calibration curve for azithromycin spanning the concentration range of 1.0 to 20 µg/mL was established to determine the concentration of unknown azithromycin analytes (Figure 3). Table 3 presents the data, including the regression equations, R, R^2, and DL. The concentration can be calculated using Beer-Lambert's law A=εbCA under these conditions. The molar absorptivity (ε) can thus be obtained from the linear equation in Table 3.

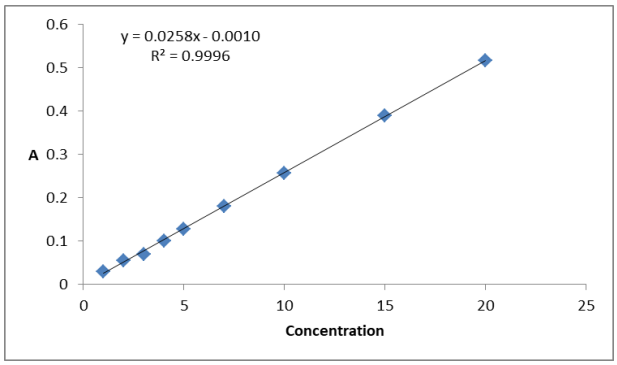

Figure 3. Azithromycin calibration curve under optimum conditions.

Table 4. Spectral parameters and statistics.

Parameter	Data
λ_{max}	415 nm
Color	Yellow
Linearity	(1.0 – 20.0) µg/mL
Molar absorptivity (ε)	20253.5 (L/mol.cm)
Regression equation	A = 0.0258x + 0.0010
Calibration Sensitivity	0.0258
Correlation of Linearity (R^2)	0.9996
Correlation coefficient (R)	0.9998
Limit of detection	0.671 µg/mL

Validation

The proposed method was validated by the analysis of five replicates for two different concentrations of each medication by working out the percentage of relative error and relative standard deviation (Table 5). The results indicate that the suggested method was valid and accurate.

Table 5. Some statistic parameters of the suggested method.

Concentration (µg/mL)		%Rel. Error	% *RSD
Taken(X)	***Measured**		
2	2.0221	+1.105	0.8721
10	9.9556	-0.444	0.6521
20	19.878	-0.161	0.6011

*Average of five measurements.

Stoichiometry of the Complexes

Using Job's method, the formation of a 1:1 complex between protonated azithromycin and the anion of BTB was confirmed (Shah et al., 2008; Taha et al., 2002), as illustrated in Figure 4. The formation of the color complex can be represented in Scheme 1.

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Continuous Variation (Job's method)

This procedure involved preparing several solutions with varying concentrations of azithromycin and the complexing agent (BTB) ($3.5x10^{-5}$ to $3.5x10^{-4}$ mmol) for each solution while maintaining a constant total volume and total moles of reactants in each mixture. However, the mole ratio of reactants systematically varies (for example, 1:9, 8:2, 7:3, etc.). In the formula VD /(VD + VR), where VD is the volume of the drug solution (azithromycin) and VR is the volume of the reagent solution, the absorbance was plotted against the volume fraction of one reactant (BTB).

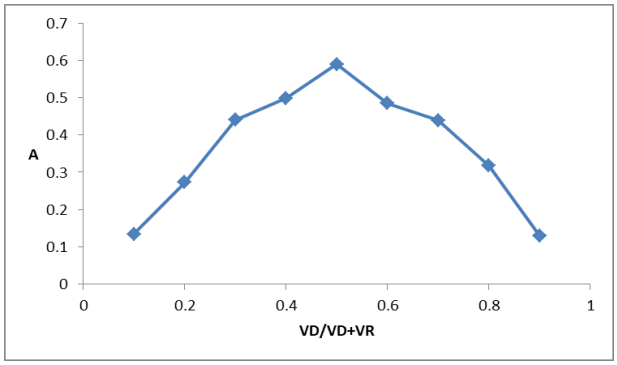
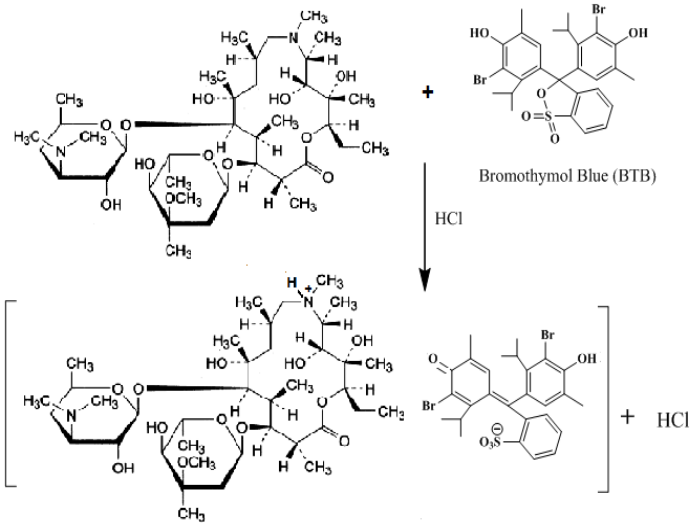


Figure 4. Continuous variation of azithromycin, BTB complex formation.



Scheme 1. The proposed reaction of the color complex.

However, there were no interferences with azithromycin determination when 250 µg/mL of the excipients mentioned in Table 6 were present.

Table 6. Recoveries of interferences with 10 µg/mL of azithromycin.

Interferences	Conc. Measured (µg/mL)	%Rec.
Sucrose	9.9424	99.424
lactose	9.9333	99.333
starch	10.211	102.11
Glucose	9.8881	98.881
Sodium Citrate	9.9635	99.635

Analysis of Dosage Dorms

The relevance of the active component content of pharmaceutical dosage forms was assessed using the suggested method. The results in Table 6 met expectations, since the RSD was within the range of 0.96±0.2 %, with a good recovery of (98.321%-101.72%). Therefore, this method can be applied for the routine determination of azithromycin.

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Table 7. Spectrometric quantification of azithromycin in medicinal compounds with the Formation of Ion-Pair

Pharmaceutical compound	Conc. (µg/mL)		% Rel. Error	%	% *RSD
	Taken(X)	***Measured**		**Rec.**	
Azithromycin tablet 500mg (as dihydrate),	5	4.9442	-1.116	98.884	0.8871
SANDOZ, Australia	10	9.9464	-0.536	99.468	0.7218
-Zithroriv Capsule Azithromycin dihydrate 250 mg,	5	5.0513	+1.026	101.02	1.2012
Egypt	10	10.172	+1.720	101.72	1.1098
ZAHA-500 tablet	5	4.9322	-1.356	98.644	0.8991
Azithromycin dihydrate 500 mg Ajanta-India	10	9.8321	-1.679	98.321	0.9019

*Average of five measurements.

4. Conclusion

Simplex optimisation has been reported for the spectrophotometric measurement of azithromycin in bulk and dosage forms. This approach utilized ion-pair formation with BTB as a chromogenic reagent and proved to be straightforward, accurate, inexpensive, and sensitive (calibration sensitivity 0.0258). These findings clearly indicate that the proposed method is effective for the tested medications.

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