Spectrophotometric determination of azithromycin in tablets. An alternative method for application in the pharmaceutical industry

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***Abstract*– In the present research, a simple spectrophotometric method for quantifying azithromycin in tablets was validated. For this purpose, validation parameters established by the United States Pharmacopoeia (USP) were evaluated. The results show that the method is linear with a coefficient of determination of 0.9995, precise with relative standard deviations ranging from 0.18% to 0.25%, and accurate with a recovery percentage of 97.75%. Likewise, four brands of azithromycin commonly sold in the city of Arequipa, Peru, were analyzed and found to comply with the uniformity of azithromycin content of 500 mg. In conclusion, the developed method could be considered by pharmaceutical laboratories, quality control laboratories, or universities to perform routine or educational tests to quantify azithromycin in tablets.**

Keywords--Azithromycin, validation, quality, spectrophotometry, tablets.

I. Introduction

Azithromycin is a member of the macrolide family of antibiotics and is commonly used to treat respiratory bacterial infections, however, several investigations have revealed possible immunomodulatory properties which is one of its most important characteristics, leading to its application in the treatment of inflammatory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), also, it has been shown that azithromycin can directly inhibit viral load [1]. Therefore, their use is common, and new brands produced by laboratories are increasingly registered, which in many cases leads to the adulteration of these drugs. For this reason, method validation is a key element in the establishment of reference methods and is within the competence of a laboratory to generate reliable analytical records [2]. This analytical information can be used for a variety of purposes such as; for decisions involving control of a product's manufacturing process, to assess whether a product meets regulatory limits, to make decisions about legal issues, international trade, health, or environmental concerns [3]. For this reason, analytical validation is of fundamental importance in the field of Good Manufacturing Practices for pharmaceutical products, as it establishes the scientific evidence that an analytical procedure provides reliable results [4]. To validate an analytical method, different parameters such as linearity, accuracy, limit of detection and quantification, precision, and recovery, among others, must be satisfied [5].

Therefore, it is important to carry out research that not only provides clear procedures for the validation process of a method but also seeks to interpret the validation parameters adequately. For this reason, the objective of this research was to validate a spectrophotometric method to determine azithromycin in tablets. For this purpose, the linearity, sensitivity, and accuracy of the spectrophotometric method were evaluated, and subsequently, azithromycin was quantified in four brands of azithromycin most commercialized in the city of Arequipa, Peru.

II. Materials and Methods

A. Reagents and equipment

Azithromycin and hydrochloric acid purchased from Merck were used for the experiments. A 40 kHz FaithFull Ultrasonic Bath and a Thermo Scientific Genesys 150 Spectrophotometer were also used.

B. Sample selection

For the development of the method to quantify azithromycin in tablets, four brands of azithromycin sold in the city of Arequipa, Peru, were chosen. The brands are shown in Figure 1. They were named brands 1, 2, 3, and 4 since this was the name used throughout this research.

C. Method validation by spectrophotometry

Linearity

First, a stock solution of azithromycin was prepared at a concentration of 1 mg/mL. This stock solution was prepared by weighing and dissolving 10 mg (0.01 g) of azithromycin standard in distilled water and was diluted in a 10 mL beaker with 0.1 M HCl. Once dissolved, it was made up to volume, and from this stock solution, calibration solutions of 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL were prepared (Figure 2).

The 20 µg/mL solution was chosen to perform a spectrophotometric sweep from 200 to 350 nm to select the working wavelength corresponding to the peak of highest light absorption. Once the wavelength was chosen, the absorbances

of all the prepared dilutions were read, and then the equation of the line and the coefficient of determination R^2 were determined in Microsoft Excel.

Fig. 1. Azithromycin Brands

Linear regression was calculated using the least squares method to find the equation of the line plotting the azithromycin concentration versus the average absorbance since the calibration solutions were prepared in triplicate [6].

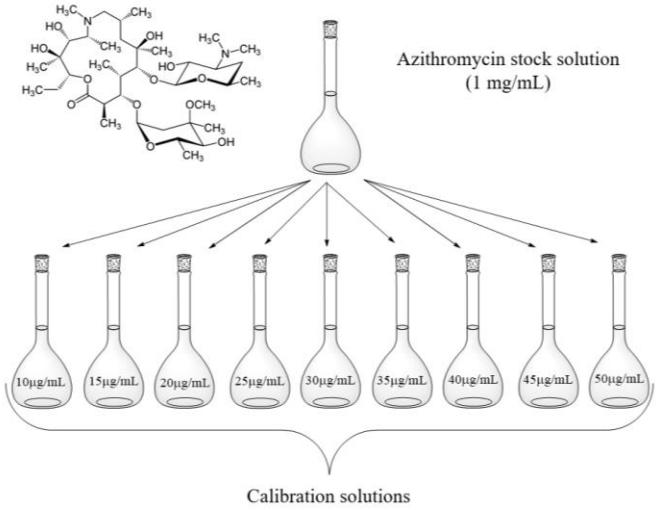

Fig. 2. Dilutions of azithromycin for the construction of the calibration curve.

$$y = a + bx \tag{1}$$

Where: (y) is the absorbance, (x) is the concentration in mg/L of azithromycin, (a) is the intercept, and (b) is the slope. Subsequently, the coefficient of determination R2 was calculated, which must be greater than 0.995 [7].

Sensitivity

Sensitivity was determined mathematically by calculating the limit of detection (LOD), which corresponds to the minimum amount of analyte (azithromycin) that the method can determine, but not quantify. This was calculated using Equation 2 [6], [7].

$$LOD = \frac{Ybl + 3(Sbl)}{b} \times \frac{1}{\sqrt{n}} \tag{2}$$

On the other hand, the limit of quantification (LOQ), which corresponds to the minimum amount of analyte (azithromycin) that the method can quantify with precision and accuracy, was calculated with Equation 3 [6], [7]

$$LOD = \frac{Ybl + 10(Sbl)}{b} \times \frac{1}{\sqrt{n}} \tag{3}$$

Where, (*Ybl*) corresponds to the intercept (*a*) of the equation of the line, (*b*) is the slope, (*n*) is the number of points on the calibration graph that were six and (*Sbl*) corresponds to the intercept of the equation of the line obtained by relating the concentration and standard deviation of the calibration graph data [6], [7].

Precision

To evaluate the precision of the method, the sample treatment process was separated into 3 steps. The first step consisted of weighing 20 tablets of each brand of azithromycin (Figure 3). Subsequently, we continued with step 2, which consisted of pulverizing in an agate mortar to avoid contamination of the sample, then we weighed the amount equivalent to 500 mg of azithromycin from the pulverized sample.

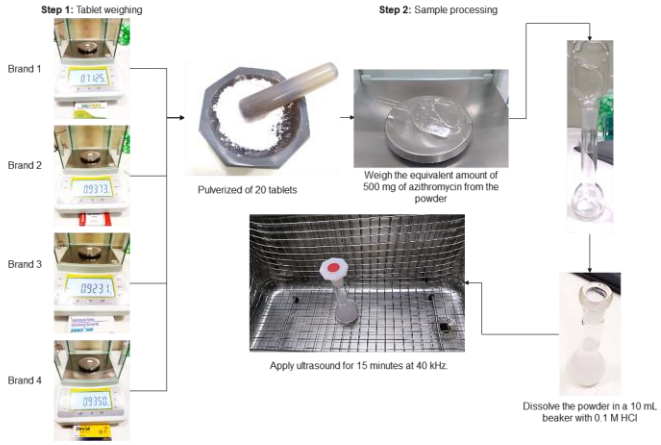

Fig. 3. Weighing, pulverizing, and tablet processing

For the weight calculation of the 500 mg equivalent amount of azithromycin in the sprayed sample for Brand 1, the 20 tablets weighed 14.2437 g (Table I), which theoretically contains 10 g of azithromycin. Then, the equivalent weight of 500 mg of azithromycin of the 20-tablet pulverized sample is 0.7122 g. Similarly, using the data in Table 1, the weights for Marks 2, 3, and 4 were calculated to be 0.9374, 0.9231, and 0.9348 g, respectively.

Once the quantities equivalent to 500 mg of azithromycin were weighed, the powder was dissolved in 0.1 M HCl in 10 mL bottles, and ultrasound was applied at 40 kHz for 15 minutes. Then, the third step (Figure 4) was carried out, where it was filtered in a 50 mL flask and made up to volume with

0.1 M HCl. Then, a dilution was made by measuring 0.2 mL of the previous solution in a 10 mL fiola, and finally, 1 mL of this last dilution was taken and diluted in 10 mL to then read this last solution in the spectrophotometer.

TABLE I
TABLET WEIGHTS OF FOUR AZITHROMYCIN BRANDS

n	Weight (g)			
	Brand 1 Xalitrox	**Brand 2 Genfar**	**Brand 3 SWAZI*500**	**Brand4 Zitrolab**
1	0.7125	0.9373	0.9231	0.935
2	0.7115	0.9376	0.9233	0.9351
3	0.7129	0.9371	0.9234	0.9344
4	0.7118	0.9369	0.9236	0.9346
5	0.7121	0.9372	0.9229	0.9353
6	0.7124	0.9367	0.9228	0.9347
7	0.7119	0.9375	0.923	0.9346
8	0.7123	0.9376	0.9226	0.9349
9	0.7125	0.9379	0.9232	0.9341
10	0.7118	0.938	0.9227	0.9352
11	0.7126	0.9377	0.9224	0.9342
12	0.7128	0.9372	0.9225	0.9351
13	0.7119	0.9368	0.9234	0.9349
14	0.7116	0.9377	0.9235	0.9347
15	0.7122	0.9378	0.9236	0.9353
16	0.7124	0.9382	0.9233	0.9354
17	0.7125	0.9372	0.9235	0.9341
18	0.7119	0.9369	0.9229	0.9348
19	0.7118	0.9375	0.9231	0.9349
20	0.7123	0.9372	0.9232	0.9351
Total (g)	14.2437	18.748	18.462	18.6964
Promedio (g)	0.71	0.94	0.92	0.93

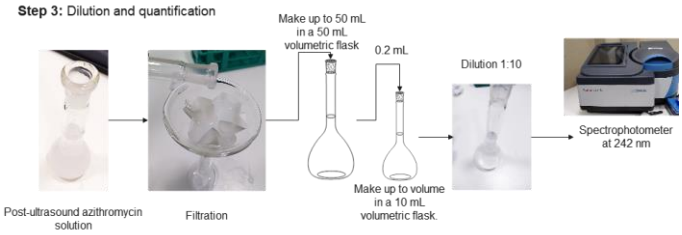

Fig. 4. Sample processing step 3

The absorbances were used to calculate the mg of azithromycin measured in the process. For this, the formula of the equation of the straight line was used with some modifications as presented below:

$$Azithromycin\,(\text{mg}) = \left(\frac{y\text{-}a}{b}\right)_{\mu g/mL} \times 50mL \times \frac{10mL}{0.2mL} \times \frac{10mL}{1mL} \times \frac{1mg}{1000\mu g} \quad (4)$$

Calculating this equation yields Equation 5, which was used for the quantification of azithromycin in tablets.

$$Azithromycin\,(\text{mg}) = \frac{y-a}{b} \times 25 \quad (5)$$

Where (y) is the absorbance, (x) is the concentration in mg/L of azithromycin, (a) is the intercept, and (b) is the slope.
The precision was expressed as the relative standard deviation (RSD) using Equation 6 [7].

$$RSD(\%) = \frac{s}{\bar{x}} \quad (6)$$

Where ($\bar{x}$) is the average of the six measurements and (s) corresponds to the standard deviation of these measurements [6], [7].

Accuracy

Finally, the accuracy was calculated by the percentage recovery method (% R), for 50 mg of standard azithromycin "*Cst*" was added before the filtrate of Step 3 of Figure 4, this solution contains an amount of enriched azithromycin "*Ce*". Once analyzed by spectrophotometry, we proceeded to calculate the *%R* using Equation 7 [6], [7].

$$\%R = \frac{Ce - C}{Cst} \times 100 \quad (7)$$

Where (C) is the concentration of azithromycin in the sprayed sample, (Ce) is the concentration spiked with (+50 mg azithromycin standard), and (Cst) is the concentration of the 50 mg azithromycin standard with which the samples were spiked in step 3 of the precision assay [6], [7].

Statistical data processing

Microsoft Excel 2019 and Prisma 6.0 software were used for statistical processing of the data.

III. RESULTS AND DISCUSSION

In response to the need to develop alternative, simple, fast, and easy-to-implement methods to determine drugs in pharmaceutical forms, the present project sought to develop a simple and fast methodology to determine azithromycin by ultraviolet spectrophotometry, considering USP guidelines to evaluate quality parameters such as linearity, precision, sensitivity, and accuracy [7].

A. Spectrophotometric scan

As a result of the present investigation, the spectrophotometric method for quantifying azithromycin using

0.1 N hydrochloric acid as solvent shows maximum UV light absorption wavelengths at 203 and 242 nm (Figure 5). The wavelength of 242 nm was chosen for the development of the method since 203 nm could present interferences with the adjuvants of the tablets, which could make the response of the method not comply with the USP specifications.

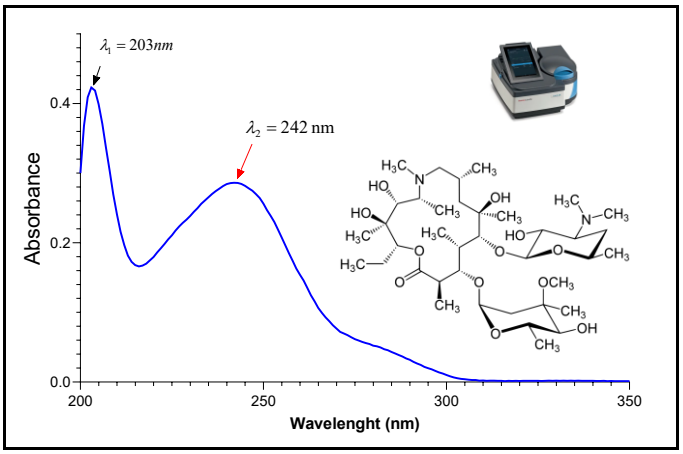

Fig. 5. Spectrophotometric scanning of 20 µg/mL of azithromycin.

In comparison with the present research, different studies validated methods to quantify azithromycin by visible spectrophotometry in tablets, such as the case of Liu *et al.* [8] who established a spectrophotometric method at 364 nm for the determination of azithromycin with 2,4-dinitrophenol, Suhagia *et al.* [9] who developed a spectrophotometric method at 412 nm for the determination of azithromycin with potassium permanganate, obtaining a yellow chromogen, Rachidi *et al.* [10] who developed a spectrophotometric method at 469 nm based on the formation of an ion pair between this drug and an orange inorganic (Mo(V)-thiocyanate) complex Paula *et al.*, [11] who proposed a method for the spectrophotometric determination at 564 nm of azithromycin using 50 mg/L quinalizarin in methanol medium, as well, Jayanna *et al.* described a spectrophotometric method at 547 nm based on the reduction of potassium permanganate in alkaline medium with azithromycin [12], Alsaab *et al.* developed a spectrophotometric technique to determine azithromycin by the oxidative coupling reaction of azithromycin by sodium periodate (SPI) and 4-amino antipyrine (AAP) producing a pink compound with an optimum absorption of 480 nm [13] and a study similar to the present one used ultraviolet spectrophotometry at 208 nm, this study was carried out by Bhimani *et al.* [14] estimated for azithromycin using phosphate buffer pH=6.8.

Unlike the present thesis, the studies developed in the visible spectrum bring additional costs for the chromophore reagents used and in the case of Bhimani et al. a phosphate buffer solution is used, which also brings additional costs, so using 0.1 N hydrochloric acid developed in this study could be a more economical and faster option.

B. Linearity Test

Regarding the validation of the method, Table II shows the results of the absorbances of the calibration solutions of concentrations of 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL of azithromycin. In this table, the absorbances obtained in triplicate are presented, as well as the averages and standard deviations.

TABLE II
LINEARITY TEST RESULTS

Azithromycin (µg/mL)	Absorbance 1	Absorbance 2	Absorbance 3	$\bar{x}$	s
10	0.12768	0.12463	0.12814	0.12682	0.0019
15	0.17642	0.17881	0.17968	0.17830	0.0017
20	0.23791	0.22973	0.23976	0.23580	0.0053
25	0.2863	0.29461	0.30138	0.29410	0.0076
30	0.35462	0.36795	0.36473	0.36243	0.0070
35	0.4189	0.40287	0.42738	0.41638	0.0124
40	0.4786	0.46729	0.47349	0.47313	0.0057
45	0.5489	0.52168	0.53108	0.53389	0.0138
50	0.60518	0.59167	0.58903	0.59529	0.0087

Plotting the concentration versus average absorbance, Figure 6 shows that the method was linear with an R^2 of 0.9995, which, according to USP, should be greater than 0.995 [7].

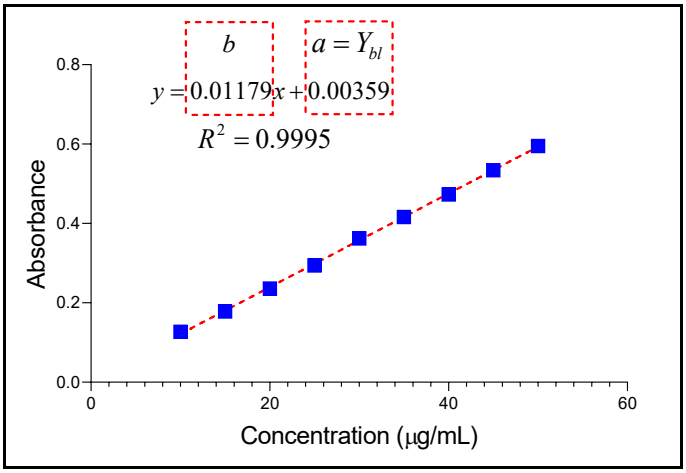

Fig. 6. Calibration curve for the quantification of azithromycin at 242 nm. Calculation of b and Ybl.

Likewise, the equation of the straight line is presented in Figure 6, and the adjustment according to the calculations of Equations 4 and 5 is shown in Equation 8, the formula for the calculation of the azithromycin concentration in tablets.

$$Azithromycin\,(\text{mg}) = \frac{Absorbance - 0.00359}{0.01179} \times 25 \quad (8)$$

C. Sensitivity test

On the other hand, for the calculation of the sensitivity of the method, it was necessary to calculate the Ybl value shown in Figure 6, where its value corresponds to 0.00359; the value of *Sbl*=0.00021 is shown in Figure 7 as a result of plotting the concentration versus the standard deviation of Table 2. The value of the slope *b*=0.01179 corresponds to the equation of the line in Figure 6. With these values, the limits of detection and quantification of the method of 0.119 and 0.161 µg/mL, respectively, were calculated (calculations are presented in Table 3.

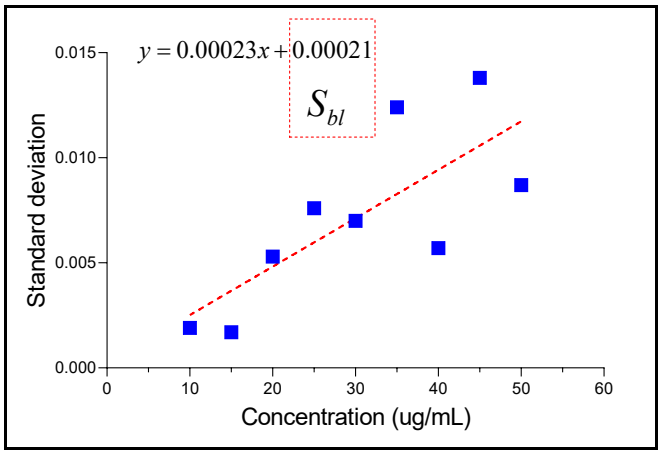

Fig. 7. Line equation for the determination of the Sbl of the method for quantifying azithromycin in tablets by spectrophotometry.

TABLE III
RESULTS OF THE SENSITIVITY ANALYSIS OF THE METHOD

LOD
$LOD = \frac{Y_{bl} + 3S_{bl}}{b} \times \frac{1}{\sqrt{n}}$ $LOD = \frac{0.00359 + 3(0.00021)}{0.01179} \times \frac{1}{\sqrt{9}}$ $LOD = 0.119 \mu g / mL$
LOQ
$LOQ = \frac{Y_{bl} + 10S_{bl}}{b} \times \frac{1}{\sqrt{n}}$ $LOQ = \frac{0.00359 + 10(0.00021)}{0.01179} \times \frac{1}{\sqrt{9}}$ $LOQ = 0.161 \mu g / mL$

D. Precision Test

To determine the precision of the method, Tables 4, 5, 6, and 7 show the RSD of brands 1, 2, 3, and 4, resulting in values of 0.25, 0.18, 0.22, and 0.18 % respectively, these values are within the specifications of the USP, which indicates that the RSD must be less than 2.7 % [7].

TABLE IV
RESULTS OF THE EVALUATION OF THE PRECISION OF THE METHOD (BRAND 1).

n	Absorbance	Azithromycin (mg)	$\bar{x}$	s	RSD (%)
1	0.2390	499.17	499.92	1.27	0.25
2	0.2397	500.66			
3	0.2395	500.23			
4	0.2389	498.96			
5	0.2403	501.93			
6	0.2387	498.54			

TABLE V
RESULTS OF THE EVALUATION OF THE PRECISION OF THE METHOD (BRAND 2).

n	Absorbance	Azithromycin (mg)	$\bar{x}$	s	RSD (%)
1	0.2398	500.87	500.37	0.88	0.18
2	0.2394	500.02			
3	0.2399	501.08			
4	0.2394	500.02			
5	0.2389	498.96			
6	0.24	501.29			

TABLE VI
RESULTS OF THE EVALUATION OF THE PRECISION OF THE METHOD (BRAND 3).

n	Absorbance	Azithromycin (mg)	$\bar{x}$	s	RSD (%)
1	0.2391	499.39	500.48	1.10	0.22
2	0.2400	501.29			
3	0.2402	501.72			
4	0.2389	498.96			
5	0.2396	500.45			
6	0.2399	501.08			

TABLE VII
RESULTS OF THE EVALUATION OF THE PRECISION OF THE METHOD (BRAND 4).

n	Absorbance	Azithromycin (mg)	$\bar{x}$	s	RSD (%)
1	0.2401	501.51	500.13	0.89	0.18
2	0.2397	500.66			
3	0.2392	499.60			
4	0.2395	500.23			
5	0.2393	499.81			
6	0.2389	498.96			

E. Accuracy test

Finally, Table 8 shows the study of the accuracy in terms of recovery, resulting in a *%R* of 97.75, this value being by the USP, which indicates that the *%R* should be between 90 and 110 %.

TABLE VIII
RESULTS OF THE EVALUATION OF THE ACCURACY OF THE METHOD AT BRAND 2

n	Sample		Standard addition (mg)	Sample + Standard addition		Recovery (%)	
	Abs	C_{Az} (mg/mL)		Abs	Concentration (mg/mL)	%R	Mean
1	0.2390	499.17	50	0.2621	548.16	97.96	97.75
2	0.2397	500.66	50	0.2629	549.85	98.39	
3	0.2395	500.23	50	0.2625	549.00	97.54	
4	0.2389	498.96	50	0.2618	547.52	97.12	
5	0.2403	501.93	50	0.2632	550.49	97.12	
6	0.2387	498.54	50	0.2619	547.73	98.39	

Abs. Absorbance, Caz: Amount of azithromycin

Other validation studies also proved to meet the validation parameters, such as the investigations of Liu et al. [8], where the R^2 was 0.9995 in the range of 5-30 µg/mL, and the mean recovery was 99.3 % the RSD was less than 0.39 %, also Suhagia et al [9]. Suhagia et al. [9], developed a spectrophotometric method at 412 nm for the determination of azithromycin with potassium permanganate obtaining a yellow chromogen, this method is linear in the range of 10-75 µg/mL, with a regression coefficient of 0.9978, Rachidi et al. achieved a linear response R^2=0.9996 a RSD of 1.07 % and found a limit of detection of 0.35 mg/L and a limit of quantification of 1.2 mg/L [11], Bhimani et al. developed a UV spectrophotometric method at 208 nm for the estimation of azithromycin using phosphate buffer pH=6. 8, resulting in a linear method in the range of 10 to 50 µg/mL with a correlation coefficient higher than 0.99, with lower limit of detection was 1.6 µg/mL and the limit of quantification was 5 µg/mL, the percentage recovery was 99.72 % [14] and Alsaab et al. developed a spectrophotometric technique to determine azithromycin by the oxidative coupling reaction of azithromycin by sodium periodate (SPI) and 4-amino antipyrine (AAP) producing a pink compound with an optimum absorption of 480 nm, resulting in a linear method with an R^2 of 0. 9998 in a range between 3 to 44 mg/L, while the limit of detection and quantification is 0.1908 and 0.5726 mg/L respectively, a relative standard deviation less than 0.645 % and an average recovery of 100.19 % [13]. In summary, the method developed in the present investigation is comparable to the methods reported so far, as they all meet the standards set by the USP for linearity, precision, and accuracy.

About the analysis of azithromycin content in the studied tablets resulted that azithromycin tablets of brands 1, 2, 3, and 4 contain 199.92 ± 1.27 mg, 500.37 ± 0.88 mg, 500.48 ± 1.10 mg and 500.13 ± 0. 89 mg which comply with the 500 mg label on each of their mediated and immediate packaging, these results are comparable to those found by Jayanna et al. in 2012, who demonstrated that the azithromycin tablets of the analyzed in their study were in good agreement with the labeled amounts [12].

V. CONCLUSION

It was possible to validate a spectrophotometric method to determine azithromycin in tablets sold in the city of Arequipa, complying with the quality parameters established by the USP, being the method linear with an R^2 coefficient greater than 0.995, precise with an RSD of less than 2.7 % and accurate with a recovery percentage close to 100 %. Likewise, it was demonstrated that the method was adequate for the determination of azithromycin in four brands, complying with the uniformity of azithromycin content of 500 mg. This method can be applied in routine quality control in developing countries.

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