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Spectrophotometric Determination of Certain Macrolide Antibiotics in Pharmaceutical Preparations

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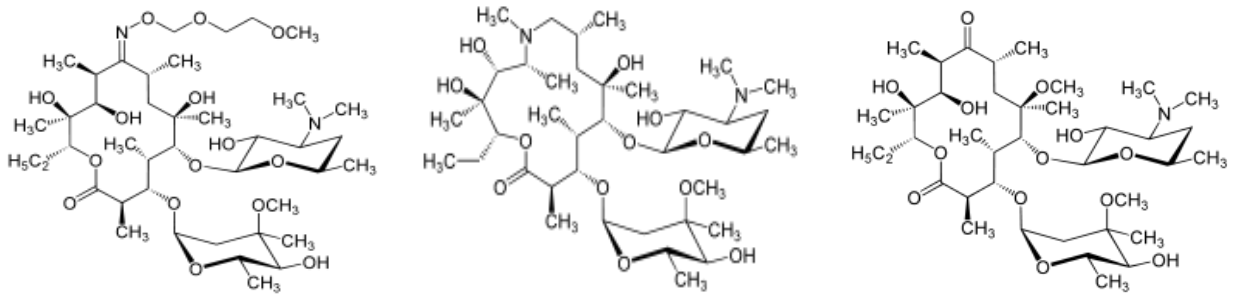
Abstract: A direct colorimetric method was described for the rapid, sensitive and accurate determination of certain macrolide antibiotics; roxithromycin (ROX), azithromycin (AZM), and clarithromycin (CLM) in bulk powder and in pharmaceutical preparations. The proposed method is based on reaction of the studied drugs with haematoxylin reagent in the presence of boric acid to give a reddish-violet chromogen λ_{max}= 598 nm. The percentage recoveries for the proposed method were 100.42±1.252, 99.59±1.134, and 99.93±1.511 within the concentration range of 0.4–8.0 μg mL^{-1}, 0.3–6.0 μg $mL^{-1}$and 0.2–4.0 μg mL^{-1} for ROX, AZM, and CLM, respectively. No interference was observed from the commonly present additives or excipients in pharmaceutical preparations. The proposed method was applied successfully for the determination of the investigated drugs in bulk powder and pharmaceutical dosage forms. The results of the analysis were found to agree statistically with those obtained with the reported methods. Furthermore, the proposed method were validated and also assessed by applying the standard addition technique.

Key words: spectrophotometry, macrolide antibiotics, haematoxylin, pharmaceutical preparations.

Introduction

Macrolide antibiotics are groups of chemically related compounds that have been isolated from actinomycetes and used primarily against Gram-positive bacteria [1]. They have a common macrocyclic lactone ring to which one or more sugars is attached and are all weak bases that are only slightly soluble in water. Macrolides and related drugs have a postantibiotic effect, that is, antibacterial activity persists after concentrations have dropped below the minimum inhibitory concentration [2]. The general structure of the studied drugs; roxithromycin (ROX), azithromycin dihydrate (AZM), and clarithromycin (CLM), are shown in Scheme 1.



ROX **AZM** **CLM**

Scheme 1. General formula and structure of the studied macrolides.

A literature search revealed different techniques for the analysis of the studied macrolides. A comprehensive review describing the analysis of macrolides[3].

Several analytical methods can be found in the literature for the studied macrolides; chromatography (HPLC) with different detectors is the most employed technique for this purpose. Hence, methods based on HPLC with tandem mass spectrometry (LC-MS/MS)[3-16], UV [17-23], fluorescence [18, 24-26], and electrochemical detection[27-29] were already reported. Capillary electrophoresis[30,31], electrochemical methods[32-39] and spectro fluorimetrically[40, 41].

Spectrphotometry methods reported for the assay of AZM include acidic hydrolysis of AZM with sulfuric acid [42], charge transfer complexation[43-47], binary complexes [48, 49], ion-pair [50, 51], Oxidation-reduction [52-53] and A flow-injection (FI) spectrophotometry by reaction of azithromycin with tetrachloro-p-benzoquinone (p-chloranil) accelerated by hydrogen peroxide and conducted in a methanol medium [54].

Spectrphotometry methods reported for the assay of ROX include charge transfer complexation[55-58], Oxidation-reduction[59-61]. ion-pair[48, 62] and UV-specrophotometric method[63, 64].

Spectrphotometry methods reported for the assay of CLM include Ion-pair[48,65-69], charge transfer complexation[70-74], Oxidation-reduction[75, 76] and UV-specrophotometric method[77,78]. Tables 1, 2 and 3 describes comparison between the reported spectrophotometric methods for determination of the studied drugs.

Table 1. Comparison between the reported spectrophotometric methods for determination of AZM.

Methods	Reagent	λ_{max}, nm	Concentration range (µg mL^{-1})	Molar absorptivity L mol^{-1} cm^{-1}	Ref.
	Sulfuric acid	**482**			**[42]**
Charge transfer complex	7, 7, 8, 8-tetracyanoquinodimethane (TCNQ)	743 842	0-3.0 x 10^{-5}	2.7×10^4 5.0×10^4	[43]
	Alizarin red in water - ethanol medium	536	(1.0 x10^{-4}- 6.0 x10^{-4}) M	1.23×10^4	[44]
	Alizarin red in alcohol-water solution	525	(5.0 x 10^{-4}- 5.5 x 10^{-3}) M	1.26×10^4	[45]
	2, 3-dichloro-5, 6-dicyano -p-benzoquinone (DDQ)	588	(5.0 x 10^{-4}- 2.25 x 10^{-3})M	2.4×10^3	[46]
	Quinalizarin	564			[47]
Binary complex	Eosin Y in aqueous buffered medium	542-544	1.0-10	8.887x10^4	[48]
	2, 4-dinitrophenylhydrazine in the presence of an acid catalyst, followed by treatment with a methanolic solution of potassium hydroxide	542–545	5.0–40		[49]
Ion pair	(Mo (V)-thiocyanate) followed by extraction with dichloroethane	469	(10^{-6} -10^{-5}) M		[50]
	wool fast blue BL Tropileon 000 nm	580 480	5-25 10-40		[51]
Oxidation -reduction	Potassium permanganate / acetyl acetone / ammonium acetate	412	10-75		[52]
A flow-injection (FI) spectroph otometry	Ferric chloride + 1,10-phenanthroline,	490	2.5-15		[53]
	Folin-Ciocalteau reagent	720	25-150		
	Tetrachloro-p-benzoquinone (p-chloranil) accelerated by hydrogen peroxide and conducted in a methanol medium	540	50 - 1600		[54]

Table 2. Comparison between the reported spectrophotometric methods for determination of RXM

Methods	Reagent	λ_{max} nm	Concentration range (µg mL^{-1})	Molar absorptivity L mol^{-1} cm^{-1}	Ref.
Charge transfer	7, 7, 8, 8-tetracyanoquinodimethane (TCNQ)	743	0-55	1.57×10^4	[55]
		844		2.93×10^4	
	Alizarin	428	0.2-18.0	1.04×10^4	[56]
	Purpurin	544	0-120	6.56×10^3	[57]
	Cresol red	456	0-80	1.05×10^4	[58]
	Marquis reagent	495	15-25		[59]
Oxidation-reduction	Potassium permanganate	412	10-75		[60]
	Ferric chloride + 1,10-phenanthroline	520	2.5 - 40		[61]
	Folin-Ciocalteu (FC)	760	2.5 - 12.45		
Ion-pair	Supracen violet 3B	590	5.0-60		[62]
	Tropaeolin 000	490	5.0-40		
	Vanillin	500	5.0-50		
	p-dimethylamino benzaldehyde (PDAB)	500			
	Eosin Y in aqueous buffered medium	542-544	1.0-10	4.814×10^4	[48]
UV-spectrophotometry		267	50-300		[63]
		205	10-150		[64]

Table 3. Comparison between the reported spectrophotometric methods for determination of CLM.

Methods	Reagent	λ_{max} nm	Concentration range (µg mL^{-1})	Molar absorptivity L mol^{-1} cm^{-1}	Ref.
Ion-association complexes	Bromothymol blue (BTB)	410	0.1-20	2.01×10^4	[65]
	Cresol red (CR)	415	2.0-20	4.378×10^3	
	Bromocresol green	415			[66]
	Tropaeolin	500	10-40	1.975×10^3	[67]
	Bromophenol blue	414	10-40		[68]
	Eosin Y	542–544	3–30	4.367×10^4	[48]
	Bromocresol green and Bromopbenol blue	414	0-60		[69]
	Concentrated hydrochloric acid and acetone	485	50-500		
Charge transfer	Purpurin in alcohol medium	548	10-150	4.49×10^3	[70]
	Marquis reagent	495	10-70		[71]
	Iodine (I_2)	363	35-135	2.986×10^3	[72]
	Tetracyanoethylene (TCNE)	420	15-95	6.877×10^3	
	alizarin	546	1-100	7.31×10^3	[73]
	quinalizarin	580	0-100	3.74×10^3	[74]
Oxidation-reduction	Ferric chloride + 1,10-phenanthroline	515	0.05-0.25		[75]
	Iron (III) + Potassium ferricyanide	740	12.5-75		[76]
	Folin-Ciocalteu reagent	775	250-125		
	2-nitrobenzaldehyde /HCl	486			[77]
UV		211	2.0-10		[78]

spectrophotometry					

Microbiological methods have been reported for the assay of these drugs [79- 81], but they suffer from many disadvantages, such as the long incubation periods and the lack of sensitivity towards other antibiotics.

The goal of the present work was to develop a simple, low-cost, efficient, rapid and sensitive colorimetric method for the quantitative determination of certain macrolide antibiotics; roxithromycin (ROX), azithromycin (AZM) and clarithromycin (CLM) in bulk and in pharmaceutical formulations using haematoxylin reagent.

Experimental

Apparatus

All absorption spectra were made using Optima UV-VIS spectrometer (SP-3000 plus) (Tokyo, Japan), and Kontron 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, both equipped with 10 mm matched quartz cells

Materials and reagents

Roxithromycin (ROX) was obtained from Obour Company for Pharmaceutical Chemical Industries (Obour, Egypt). Azithromycin (AZM) and Clarithromycin (CLM) were provided by (Shiba Pharmaceutical & Chemicals Company, Sana'a, Yemen). All standard drugs were used as received, and their solutions were stable for at least 1 week if stored in a cool place.

Standard solutions

A 100 µg mL^{-1} standard stock solution of each of the studied drugs was prepared by simple dissolution of 0.01 g of the pharmaceutical pure drug, in approximately 5.0 mL of methanol and further dilution to the mark with bidistilled water in a 100 mL volumetric flask. Working standard solutions were prepared from suitable dilution of the standard stock solution.

Pharmaceutical preparations

Roxicin tablets (Obour pharmaceutical com., Egypt), labeled to contain (300 mg ROX / tablet). Roxid tablets (T3A, Assuit, Egypt), labeled to contain (300 mg ROX/ tablet).

Azithrocine tablets (Alpha, Aleppo Pharmaceutical Industries, Aleppo-Syria), labeled to contain (500 mg AZM/ tablet). Xithrone tablets (Amoun Pharmaceutical Industries Company, Cairo, Egypt), labeled to contain (500 mg AZM/ tablet). Zisrocin capsules (EgyPharm, 6th of October, Egypt) labeled to contain (500 mg AZM/ capsule).

Claribiotic tablets (Amirya pharmaceutical Industries, Alexandria, Egypt), labeled to contain (500 mg CLM / tablet) B.N. Klarimix tablets (Sigma Pharmaceutical Industries (Quasna, Egypt) labeled to contain (500 mg CLM/ tablet). Klacid ® suspension (Galaxo Wellcome, Cairo, Egypt) under license of Abbott Laboratories International), labeled to contain (250 mg CLM / 5 mL).

Reagents

Haematoxylin (Aldrich Chemical Co. Ltd, England), freshly prepared daily by dissolving 100 mg in 2.0 mL of 0.5 % boric acid and completing the volume to 50 mL with bidistilled water. It should be stored in a cool place in a tightly stoppered dark glass bottle.

General assay procedure

One mL of each standard or sample preparation was pipetted into a series of dry 10-mL volumetric

flasks, 1.0 mL haematoxylin reagent (2.0 mg mL^{-1}) was added, allowed to stand for 30 min at 25 ±5 °C, and 1.0 mL of 0.05 % boric acid was added. After completion to the mark with bidistilled water, the absorbance was measured at 598nm against a reagent blank similarly treated. The calibration curves were constructed and the regression equations were computed (Table 1).

IV. Analysis of Pharmaceutical Formulations

Procedure for tablets

The contents of ten tablets were removed and finely powdered using an agate mortar. The combined contents were mixed and weighed accurately. A portion of the powder equivalent to 50 mg of the drug was accurately weighed and exactly 25 mL of methanol was added, sonicated for about 20 min, left for a time in a refrigerator to allow any insoluble matter to settle down and then filtered into a 50 mL volumetric flask. The solution was then completed to volume with bidistilled water. Working standard solutions were prepared from suitable dilution of the standard stock solution and the procedure was completed as described for preparing the calibration graph. The nominal contents of the tablets were determined either from the calibration graph or using the corresponding regression equation.

Procedure for oral suspension

An accurately measured volume of the freshly reconstituted oral suspension equivalent to 50 mg of the drug was extracted with 25 mL of methanol, sonicated for about 20 min, left for a time in a refrigerator to allow any insoluble matter to settle down, and filtered into a 50 mL volumetric flask. The solution was then completed to volume with bidistilled water. Working standard solutions were prepared from suitable dilution of the standard stock solution and the procedure was completed as described for preparing the calibration graph. The nominal contents of the suspension were determined either from the calibration graph or by using the corresponding regression equation.

Stoichiometric relationship

The stoichiometric ratios of the charge transfer complexes formed between the studied drugs under investigation and haematoxylin reagent were determined by applying the continuous variation method attributable to Job and modified by Vosburgh and Coober [82] at the optimum wavelengths of maximum absorbance. The reagent was mixed in various proportions with drug and diluted to volume in a 10 ml calibrated flask with bidistilled water following the above mentioned procedures.

Results and Discussion

Three certain macrolide antibiotics; ROX, AZM, and CLM, were found to react with haematoxylin reagent. The reaction was carried out in the presence of boric acid to produce a reddish-violet color. The absorption spectra of the reaction products of all the studied drugs were identical and exhibited maximum absorption at 598 nm but with different absorptivities. Figure 1. shows the absorbance spectra of the three investigated drugs, haematoxylin and their reaction products. The drug and the reagent have no absorption at 598 nm.

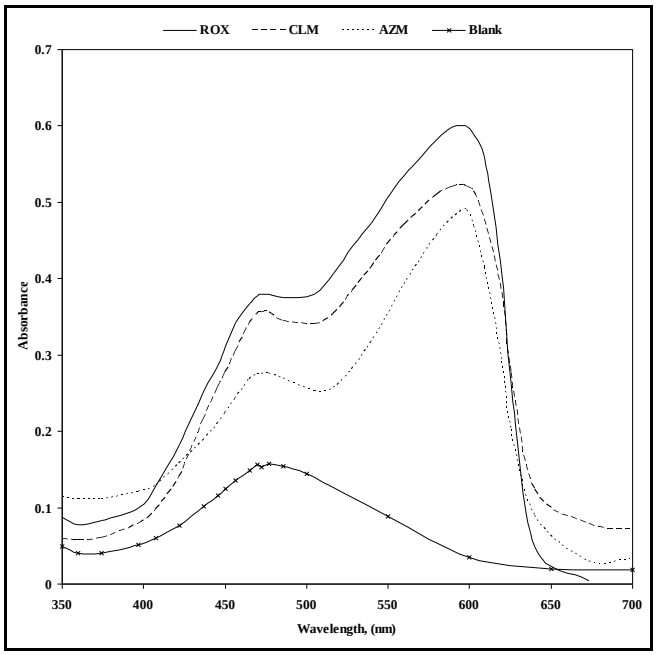


Figure 1. Absorption spectra of the studied drugs (4.0 µg mL^{-1}) against haematoxylin (0.2%) in presence of 0.05% boric acid.

Factors affecting the color development and sensitivity were studied and optimized.

Effect of boric acid concentration

Haematoxylin is oxidized slowly and spontaneously (the color changes from yellow to red), so attempts were made to stabilize it. Two mL of 0.5 % boric acid per 50 mL of the reagent was found satisfactory for optimum stability of the reagent, forming haematoxylin borate.

The absorbance readings attained a maximum within 30 min. After this time, there was a continuous increase in the absorbance readings but with a small rate. Therefore, 1.0 mL of 0.05 % boric acid was added for quenching the reaction, Figure 2. Taken ROX with haematoxylin as example; excessive amounts of boric acid cause a decrease in the absorbance readings.

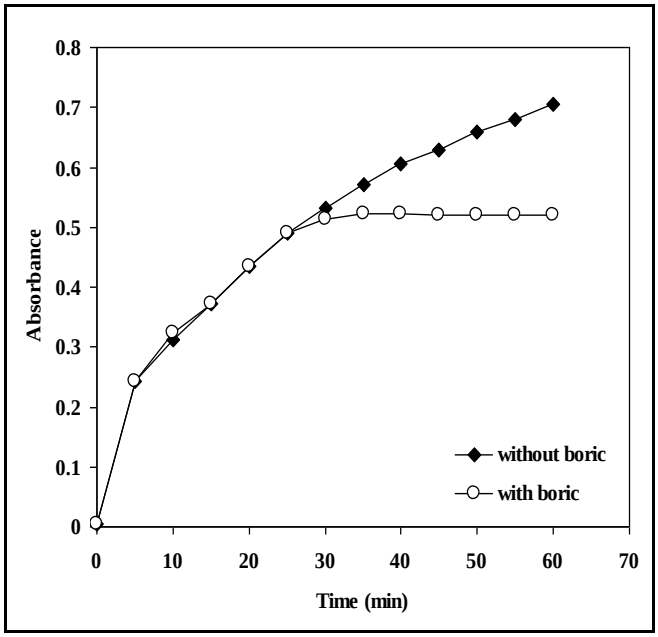


Figure 2. Effect of development time on intensity of the colored product of haematoxylin with ROX (4.0 µg mL^{-1}) with (1) boric acid and (2) without boric acid.

Effect of Solvent

The solvents studied were water, methanol, ethanol, n-propanol, iso-propanol and acetone. The use of water afforded maximum sensitivity and color stability. Other halogenated and water immiscible solvents were unsuitable as the formed colored product, being polar, is not extractable with them.

Effect of reagent

One mL of 0.2 % solution of haematoxylin reagent was found to be sufficient for maximum color intensity. Increasing the reagent concentration did not affect the color intensity (Figure 3).

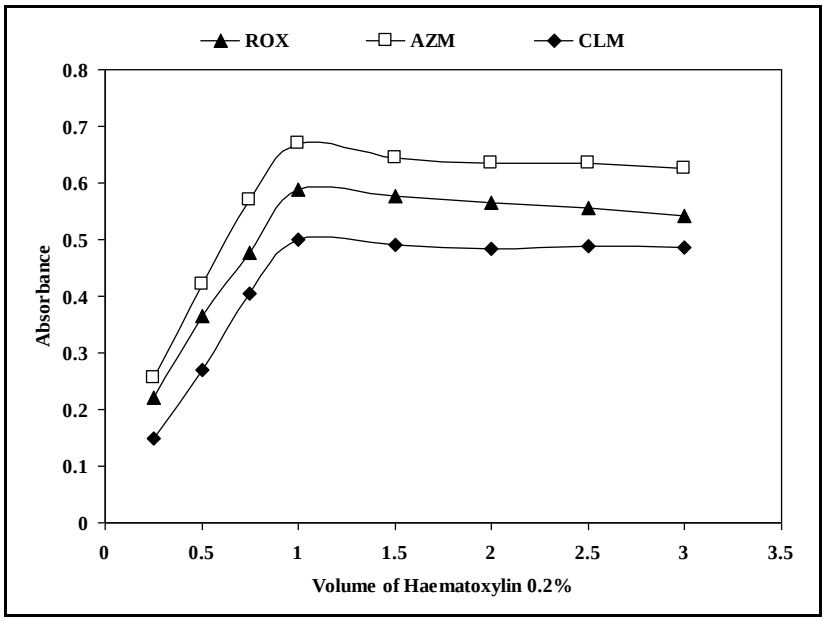


Figure 3. Effect of volume of haematoxylin 0.2% reagent on intensity of the colored product of with the studied drugs (4.0 µg mL^{-1}) and 1.0 mL 0.05% boric acid, at λ_{max}=598 nm.

Stoichiometric ratio

The molar ratios were determined by studying the continuous variation method between the studied drugs and haematoxylin in the presence of 0.05 % boric acid revealed 1 : 1 ratio. Acidic compounds did not produce such chromogenic products and they have a marked decolourizing action on the formed chromogen [83].

Validation of the proposed method

Linearity, detection, and quantitation limits

Following the proposed experimental conditions, the relationship between the absorbance and concentration for each studied drug was quite linear in the concentration range 0.4–8.0, 0.3–6.0 and 0.2–4.0 µg mL^{-1} for ROX, AZM, and CLM, respectively. The regression equations were derived using the least-squares method [84]. The intercept (a), slope (b), correlation coefficient (r), molar absorptivities (ε), and sandell sensitivity values for all studied macrolides are summarized in Table 4.

Table 4. Statistical analysis of calibration graphs and analytical data for determination of the studied drugs using the proposed method compared with the reported method [48].

Parameters	ROX	AZM	CLM
Wavelengths λ_{max} (nm)	587	589	587
Beer's law limits (µg mL^{-1})	0.4 - 8.0	0.3 - 6.0	0.2 - 4.0
Ringboom limits (µg mL^{-1})	0.7 - 0.75	0.5 - 5.5	0.5 - 3.7
Molar absorptivity ε, (L/mol^{-1} cm^{-1}) x 10^5	0.935	1.1843	1.552
Sandell's Sensitivity (ng cm^{-2})	8.95	6.63	4.82
Regression equation [a]			
Slope (b)	0.1067	0.1550	0.1976
Intercept (*a*)	0.0053	- 0.0036	0.0054
Correlation coefficient (*r*)	0.9998	0.9998	0.9994
Detection limits LOD (µg mL^{-1})	0.0872	0.0561	0.0425
Quantification limits LOQ (µg mL^{-1})	0.291	0.187	0.142
RSD%	1.24	1.139	1.5126
RE%	1.30	0.986	1.588
Mean recovery % [b]	100.42	99.59	99.93
± Standard Deviation	1.252	1.134	1.511

Variance	1.567	1.285	2.285
t-test [c]	1.37 (2.20)	0.508 (2.18)	0.295(2.20)
F-ratio [c]	1.85(4.95)	1.91 (3.97)	2.64 (4.95)

[a] A = a + b C, where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drug in µg ml^{-1}. LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity coefficient.
[b] Mean ± standard deviation of six determinations.
[c] Values in parentheses are the tabulated *F*- and *t*-values at *P* = 0.05.

The percentage recoveries of the pure drugs using the proposed method compared with that given by the reported method [48] are illustrated in Table 4. The validity of the proposed method was evaluated by statistical analysis [85] between the results achieved from the proposed method and that of the reported method [48]. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 4), there is no significant difference between the proposed and reported method regarding accuracy and precision.

The detection limit (LOD) is defined as the minimumlevel at which the analyte can be reliably detected for the 3 drugs was calculated using the following equation [85] and listed in Table 4:

LOD = *3s / k*

where *s* is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and *k* is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, the detection limits were found to be 0.0872, 0.0561 and 0.0425 µg mL^{-1} for ROX, AZM and CLM, respectively.

The limits of quantization, LOQ, is defined as the lowest concentration that can be measured with acceptable accuracy and precision [85]

LOQ = *10 s / k*

According to this equation, the limit of quantization was found to be 0.291, 0.187 and 0.142 µg mL^{-1} for ROX, AZM and CLM, respectively.

Accuracy and precision

Percentage relative standard deviation (RSD%) as precision and percentage relative error (Er %) as accuracy of the proposed spectrophotometric method were calculated. Precision was carried out by analyzing six samples of each of the studied macrolides at four different concentration levels. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the suggested method (Table 5). The percentage relative error calculated using the following equation:

Er % = [(founded – added) / added] x 100

The inter-day and intra-day precision and accuracy results are shown in (Table 5). These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

Table 5. Intra-day and Inter-day accuracy and precision data for the proposed method on pure sample of the investigated drugs.

Drug	Taken (µg mL^{-1})	Intra-day				Inter-day			
		Recovery % [a]	Precision RS D %	Accuracy Er %	Confidence limit	Recovery % [a]	Precision RS D %	Accuracy Er %	Confidence limit
ROX	1.0	99.65	0.54	-0.35	0.9965±0.0022	100.25	0.50	0.25	1.0025±0.002
	2.0	100.15	0.63	0.15	2.003±0.0052	99.95	0.46	-0.05	1.999±0.0038
	4.0	99.80	0.45	-0.20	3.992±0.0073	99.70	0.68	-0.30	3.988±0.011
	6.0	99.90	0.41	-0.10	5.994±0.010	99.85	0.67	-0.15	5.991±0.016
Mean± SD		99.88 ± 0.21				99.94 ± 0.23			
AZM	1.0	100.20	0.64	0.20	1.002±0.0026	100.10	0.49	0.10	1.001±0.002

	2.0	100.05	0.51	0.05	2.001±0.0042	99.80	0.53	-0.20	1.996±0.0043
	4.0	100.10	0.58	0.10	4.004±0.0095	99.75	0.58	-.25	3.99±0.0094
	5.0	99.80	0.39	-0.20	4.99±0.0079	100.20	0.49	0.20	5.01±0.010
Mean± SD		100.04 ± 0.17				99.96 ± 0.22			
CLM	0.5	99.95	0.56	-0.05	0.4998±0.0011	100.10	0.44	0.10	0.5005±0.0009
	1.0	100.30	0.47	0.30	1.003±0.0019	99.90	0.62	-0.10	0.999±0.0025
	2.0	100.15	0.55	0.15	2.003±0.0045	100.30	0.64	0.30	2.006±0.0052
	3.0	99.80	0.45	-0.20	2.994±0.0055	100.40	0.53	0.40	3.012±0.0065
Mean± SD		100.05 ± 0.22				100.18 ± 0.22			

[a] Mean of six determination. RSD%, percentage relative standard deviation; Er%, percentage relative error.

Recovery studies

To confirm the accuracy of the method, recovery studies were performed by using the point standard addition method [(84)]. This depends upon the addition of a known quantity of the standard macrolide antibiotics to a fixed amount of the corresponding pharmaceutical sample equivalent to about 1.0 µg macrolide antibiotics, and then analyzing the resulting solution by the proposed spectrophotometric method. The difference in absorbance of standard and sample plus standard was used to calculate the concentration of sample after each addition. Results indicate good recoveries (99.08–100.08% ± 0.37–0.83) and prove the lack of interference due to common excipients and, hence, accuracy of the proposed method (Table 6).

Interference studies

The selectivity of the proposed spectrophotometric method was investigated by observing any interference encountered from some common excipients of the pharmaceutical formulations such as starch, lactose, sucrose, glucose, gum acacia, and magnesium stearate. It was shown that these excipients did not interfere with the proposed method. So, The proposed method is able to determine the analyte in the presence of common excipients.

Ruggedness and robustness

The ruggedness of the proposed method was assessed by applying the procedures using 2 different instruments (described in the *Experimental* section) in 2 different laboratories at different times. Results obtained from laboratory-to-laboratory and day-to-day variation were found to be reproducible because the RSD did not exceed 2%. Robustness of the procedures was assessed by evaluating the influence of small variation of experimental variables, i.e., concentrations of reagent and reaction time, on the analytical performance of the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results. This provided an indication of the reliability of the proposed method during routine work.

Application of the proposed method to analysis of pharmaceutical formulations

The proposed method was successfully applied to the determination of the 3 macrolides in their pharmaceutical formulations (Table 6). The results were compared statistically, by applying the *t*- and *F*-tests, with the results obtained by the reference method [(48)]. The results obtained by the proposed method revealed no significant difference were found between the calculated and theoretical values of both the proposed and reference methods at 95% confidence level. This indicated similar accuracy and precision in the analysis by the proposed and reported methods. It is evident from these results that the proposed method are applicable to the analysis of the studied drugs in its bulk form and in pharmaceutical formulations with comparable analytical performance. The critical recommendations of some of these methods might be based on their relative sensitivities (depending upon the amount of specimen available for analysis) and experimental conditions (reaction time, reagent volume, etc.).

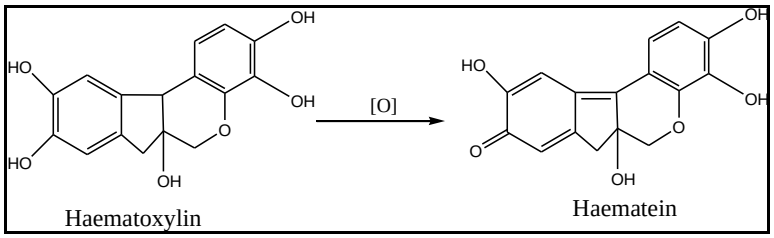
Table 6. Determination of the studied drugs in their pharmaceutical preparations applying the standard addition technique.

	Taken (µg mL^{-1})	Added (µg mL^{-1})	Proposed method	Reference method	Proposed method	Reference method	Proposed method	Reference method
			Recovery % [a]		Recovery % [a]		Recovery % [a]	
			Roxicin tablets (300 mg RXM/ tablet)		Roxid tablets (300 mg RXM/ capsule)			
	1.0	-	99.80	98.95	98.65	98.30		
		2.0	99.35	99.55	99.20	99.10		
		4.0	99.60	100.0	99.45	99.85		
		6.0	100.10	99.70	99.90	99.30		
Mean ± SD			99.69 ± 0.44	99.58 ± 0.48	99.22± 0.52	99.14± 0.64		
t-Value [b]			0.29		0.17			
F-value [b]			1.19		1.51			
			Azithrocine tablets (500 mg AZM/ tablet)		Xithrone tablets (500 mg AZM/ tablet)		Zisrocin capsules (500 mg AZM/ capsule)	
	1.0	-	98.60	98.60	99.20	99.05	98.40	99.10
		2.0	99.80	100.15	99.95	98.80	99.55	99.75
		3.0	100.25	99.25	100.30	98.50	100.10	98.90
		4.0	100.1	99.50	98.90	99.95	100.20	100.35
Mean ± SD			99.66 ± 0.80	99.38 ± 0.64	99.59 ± 0.65	99.08 ± 0.63	99.56± 0.83	99.53± 0.66
t-Value			0.473		0.975		0.049	
F-value			1.56		1.06		1.58	
			Clarimax® tablets (250 mg CLM/ tablet)		Claritop® tablets (250 mg CLM/ tablet)		Klarimix tablets (500 mg CLM/ capsule)	
	1.0	-	99.60	100.67	99.55	99.78	99.47	99.63
		1.0	100.56	99.42	100.12	98.78	99.45	99.31
		2.0	100.11	99.95	100.15	99.43	98.68	98.23
		3.0	98.80	100.28	100.65	100.02	99.12	98.56
Mean ± SD			99.77 ± 0.75	100.08 ± 0.53	100.12 ± 0.45	99.53 ± 0.51	99.18± 0.37	99.13± 0.43
t-Value			0.585		1.50		0.15	
F-value			2.00		1.28		1.35	

[a] Average of at least 3 determinations.
[b] The tabulated Values of *F*- and *t*-values at (*P* = 0.05) are 9.28 and 3.18, respectively.

Suggested reaction mechanism

Haematoxylin was reported [86] as the most widely used and versatile dye in histological technique and was used in stains for cell nuclei. For these purposes haematoxylin was oxidized to haematein. The hydroxyl group at position 9 is oxidized to the corresponding keto derivative and a conjugated system will be formed causing the observed bathochromic schift. Haematoxylin is first oxidized to haematein in the solution at the concentration used, there is always enough dissolved oxygen present to form sufficient haematin. This oxidation is more rapid in alkali [(87)]. The ionized haematein will possess two possible resonance forms and these account for the intense color of the ionic solution at 598 nm, Scheme 2.



Haematoxylin Haematein

Scheme 2. Oxidation of Haematoxylin to haematein

Conclusion

The present proposed spectrophotometric method developed accurate, sensitive, and simpler than the reference methods. It should be useful for reliable and practical quality control analysis of the studied macrolide antibiotics in pure and in pharmaceutical formulations without interference from common additives. The proposed method is superior to the previously reported methods in terms of simplicity and sensitivity.

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