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Bromometric Estimation of Cefixime, Clarithromycin and Clindamycin in Bulk and Dosage Forms

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ABSTRACT

Two spectrophotometric methods are described for determination of Clarithromycin, Clindamycin and Cefixime in bulk and pharmaceutical dosage forms using insitu generated bromine as oxidizing agent and either methylene blue or methyl orange as chromogenic agents. Drugs are treated with known excess of bromine and residual unreacted bromine is determined by treating with fixed amount of either methylene blue or methyl orange then measuring absorbances at (666nm for clindamycin and cefixime or 678 nm for clarithromycin) and 510 nm respectively. The amount of bromine reacted corresponds to the amount of each drug.The effects of acidity, bromate-bromide volume and time, on the absorption were studied. Calibration curves were linear over ranges of 3.2–16 $\mu g.ml^{-1}$ for Clarithromycin,1.6- 4.8 $\mu g.ml^{-1}$ for Clindamycin, 0.8-7.2 $\mu g.ml^{-1}$ for Cefixime in case of methylene blue and of 6.4–19.2 $\mu g.ml^{-1}$ for Clarithromycin, 0.8-4.0$\mu g.ml^{-1}$ for Clindamycin, 0.4-2 $\mu g.ml^{-1}$ for Cefixime in case of methyl orange. The methods were satisfactory applied for the determination of drugs in both bulk and pharmaceutical dosage forms and results were compared statistically with reference methods.

Key words: *Clarithromycin, Clindamycin, Cefixime, Methylene blue and Methyl orange,* Spectrophotometry

INTRODUCTION

Cephalosporins, like all β-lactam antibiotics, inhibit bacterial growth by interfering with a specific step in bacterial cell wall synthesis (katzung, 2001). Cephalosporins consist of a fused β-lactam-A -dihydrothiazine two-ring system, known as 7-amino cephalosporanic acid (7-ACA) and vary in their side chain substituents at C_3 (R_2), and C_7 (acylamido, R1) (Van Krimpen *et al.*, 1987) . In this study Cefixime was determined spectrophotometically. Several methods have been developed for its determination, including spectrophotometric methds (Walily *et al.* 2000; Almomani *et al.*, 2001; Elbashir *et al.* 2011; Naimul *et al.*, 2013), high-performance liquid chromatography (HPLC) (Vladimirov *et al.*, 1957; Vladimirov *et al.*, 1988; Zhong *et al.*, 2005; Katlan *et al.*, 2009; Khan *et al.*, 2011), Electro chemical methods (Kakehi et al., 1999; Memon *et al.*, 2007).

Macrolides are a group of drugs (typically antibiotics) whose activity stems from the presence of a macrolide ring, a large macrocyclic lactone ring to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. The lactone rings are usually 14-, 15-, or 16-membered. The mechanism of action of macrolides is inhibition of bacterial protein biosynthesis, and they are thought to do this by preventing peptidyltransferase from adding the peptidyl attached to tRNA to the next amino acid (Martin *et al.*, 2003) (similarly to chloramphenicol as well as inhibiting ribosomal translocation.

From this group we study clarithromycin.. several methods have been developed for its determination, including specrophotometric methds (Chowdary *et al.*, 2003; Mohamed *et al.*, 2007; Suraga *et al.*, 2008; Rania *et al.*, 2012), high-performance liquid chromatography (HPLC) (Jiang *et al.*, 2007; Weili *et al.*, 2007; Willemic *et al.*, 2009) electro chemical methods (Flurer *et al.*, 1996) .

Lincosamide antibiotics are one of the classes of antibiotics most associated with pseudomembranous colitis caused by Clastridium difficile. Lincosamides prevent bacteria replicating by interfering

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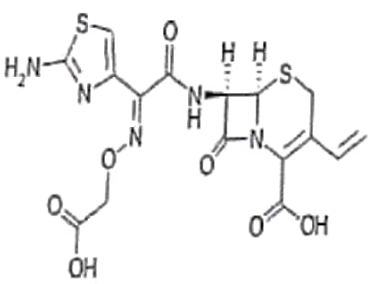
with the synthesis of proteins. They bind to the 23s portion of the 50S subunit of bacterial ribosomes and cause premature dissociation of the peptidyl-tRNA from the ribosome (Martin *et al.*, 2003). From this group we study clindamycin. Several methods have been developed for its determination, including specrophotometric methds (El-Yazbi *et al.*, 1993; Barazendeh *et al.*, 2013), high-performance liquid chromatography (HPLC) (Fieger *et al.*, 1999; Martenas *et al.*, 2001; Batzias *et al.*, 2004), Electro chemical methods (Wang *et al.*, 2008).

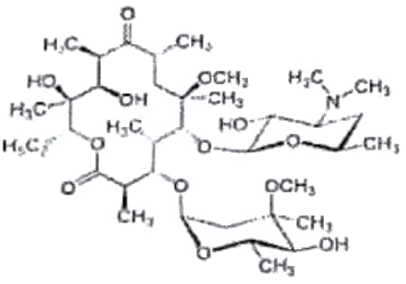
Redox reactions are employed in determination of organic cations and anions as well as organic substances. They have also been used as indicator reaction for kinetic catalytic methods. In redox reactions, the reaction products include the oxidized (or reduced) form of the analyte and the reduced (or oxidized) form of the reagent. Change in the absorbance of one of the reactants or products, induced by the reaction, can be employed in the determination.

An example of which one is the oxidation of the analyte by reagent (bromine) and then excess regent is determined using other spectrophotometric reaction (such as oxidation of methylene blue, or methyl orange by excess bromine followed by determination of residual dye) .

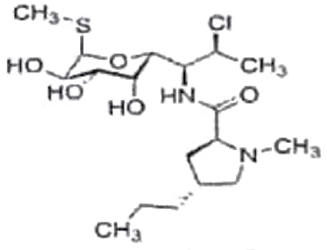
In this study, Cefixime, Clarithromycin and Clindamycin have been determined spectrophotometrically through indirect redox method depending on oxidation of drug by insitu generated bromine and evaluation of excess bromine by using either methylene blue or methyl orange.



Cefixime



Clarithromycin



Clindamycin

MATERIALS and METHODS

Apparatus

By dissolving pure drug in 100 ml with bidistilled water. Clarithromycin and Cefixime (Sigma). Standard solution (Clarithromycin) 13.7 $\mu g.ml^{-1}$ & 7.8 $\mu g.ml^{-1}$ (Cefixime) for Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1 cm quartz cells connected to windows compatible computer using UV Win 5 Software v5.0.5(USA).

Materials and reagents

All solvents and reagents were of analytical grade and double bidistilled water was used throughout the work. Clindamycin HCl (Sigma) Standard solution 7.63 $\mu g.ml^{-1.}$ For molar ratio $1.8x10^{-3}$ M were prepared molar ratio $1.8x10^{-3}$ M were prepared by dissolving pure drug in 15ml of methanol then completing to 100 ml with bidistilled water. 5 M HCl (El-Nasr Chemicals, Egypt) was prepared by diluting 225. ml of concentrated HCl (36%) to 500 ml. Methylene Blue & Methyl Orange 60 $\mu g/ml$ for molar ratio $1.8x10^{-3}$ M (Universal Fine Chemicals, India) were dissolved in 20 ml methanol then completed to 100 ml with bidistilled water (stable for 2 weeks at least). Bromate /

ISSN 1110-5089

Bromide stock solution was prepared by dissolving 0.1 gm of potassium bromate (Winlab, England) and 1.0 gm of potassium bromide (Winlab, England) in 100 ml bidistilled water (stable for 10 days at least). Working solution was freshly prepared daily by diluting 2.5 ml of stock solution to 100 ml with bidistilled water (25 µg/ml in case of methylene blue), 1.25 ml of stock solution (12.5 µg/ml in case of methyl orange)

Pharmaceutical preparations

The following available preparations were analyzed: Clindam ® tablets labeled to contain 150 mg Clindamycin per tablet. Batch No. 11318 (Sigma, Egypt), Ximacef ® capsules labeled to contain 400 mg Cefixime HCl per capsule. Batch No. 1240009 (Sigma, Egypt),Clarihro ® tablets labeled to contain 250 mg Clarithromycin per tablet. Batch No. 502102 (Amriya, Egypt).

General spectrophotometric procedures and construction of calibration curves using Methylene Blue method

To working solution in 10 - ml volumetric flasks, (0.2 – 6) ml (in case of clindamycin solution) , (0.2-1) ml (in case of clarithromycin) and (0.1-0.9) ml (in case of cefixime) add 1 ml bromate - bromide (cefixime and clindamycin) and 0.8 ml (in case of clarithromycin) then acidify using 0.2 ml 5 M HCl (in case of clindamycin and clarithromycin) and 0.8 ml (in case of cefixime) , close flasks and stand for 10 minutes, add 1 ml dye working solution then stand for another 10 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 678 nm (in case of claritromycin) and 666 nm (in case of clindamycin and cefixime).

General spectrophotometric procedures and construction of calibration curves using Methyl Orange method:

To working solution in 10 - ml volumetric flasks, (0.1 – 0.5) ml (in case of clindamycin HCl) or (0.2 – 1) ml (in case of clarithromycin) and (0.1-0.5) ml (in case of cefixime) add 1 ml bromate - bromide then acidify using 0.6 ml (in case of clindamycin and cefixime) or 0.2 ml (in case of clarithromycin) 5 M HCl, close flasks and stand for 10 minutes except in case of clindamycin (5 ninutes) add 1 ml dye working solution then stand for 2 minutes and complete to mark with bidistilled water then measure absorbance against reagent blank at 510 nm.

Procedures for pharmaceutical preparations

Clindam

10 tablets were weighed and powdered. An accurately amounts of the powder equivalent to76.3 mg of clindamycin were extracted in bidistilled water, filtered into 100-ml measuring flask and completed to volume with bidistilled water to give a final concentration of 7.63 $\mu g.ml^{-1}$ .The procedures were then completed as previously mentioned under the general procedures.

Clarithro and Ximacef: 10 tablets and 10 capsules were weighed and tablets were powdered. An accurately amounts of the powder equivalent to 137 mg of clarihtomycin and 78 mg of cefixime were disolved in 15 ml of methanol, filtered and washed with methanol into 100-ml measuring flask and completed to volume with bidistilled water to give a final concentration of 13.7 $\mu g.ml^{-1}$ of clarihtomycin and 7.8 $\mu g.ml^{-1}$ of cefixime. The procedures were then completed as previously mentioned under the general procedures.

RESULTS and DISCUSSION

The proposed spectrophotometric methods are indirect and based on the determination of the residual bromine (*in situ* generated) after allowing the reaction between each drug and a measured amount of bromine to be complete. The xss bromine was determined by reacting it with a fixed amount of either methylene blue or methyl orange dye. The methods rely on the bleaching action of bromine on

the dyes due to oxidative destruction of these dyes as shown in figure (1) (in case of methylene blue). ClindamycinHCl ,clarithromycin and cefixime when added in increasing amounts to a fixed amount of insitu generated bromine, consume the latter proportionately with a concomitant fall in the concentaration of bromine. When a fixed amount of dye is added to the decreasing amounts of bromine, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of each drug.The insitu generation of bromine is carried out using a mixture of potassium bromate and potassium bromide in presence of 5 M HCl according to the following equation (Tharpa and Basavaiah, 2009) :

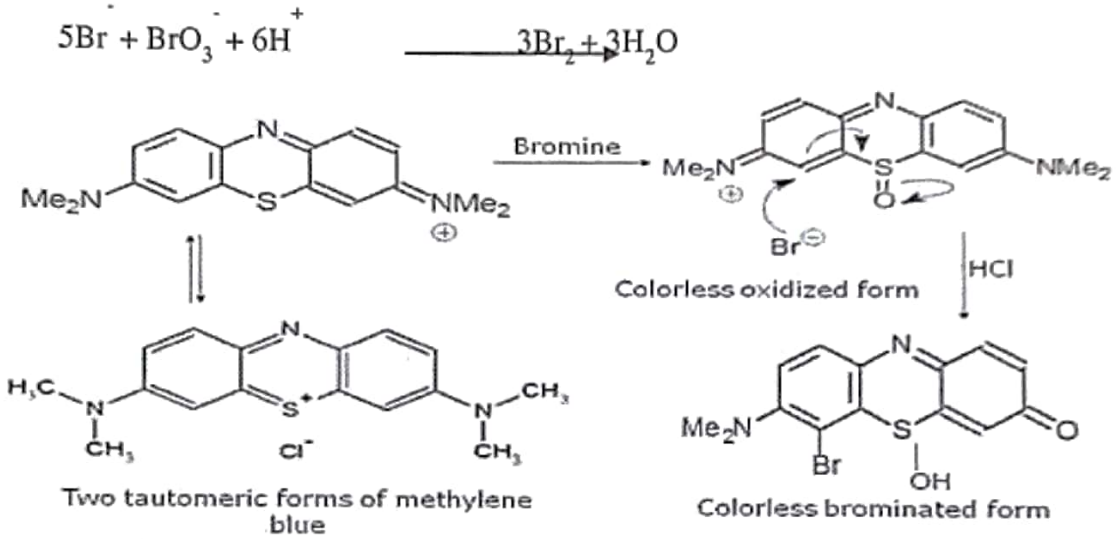


Figure 1. Proposed structures of different forms of methylene blue before and after bromination

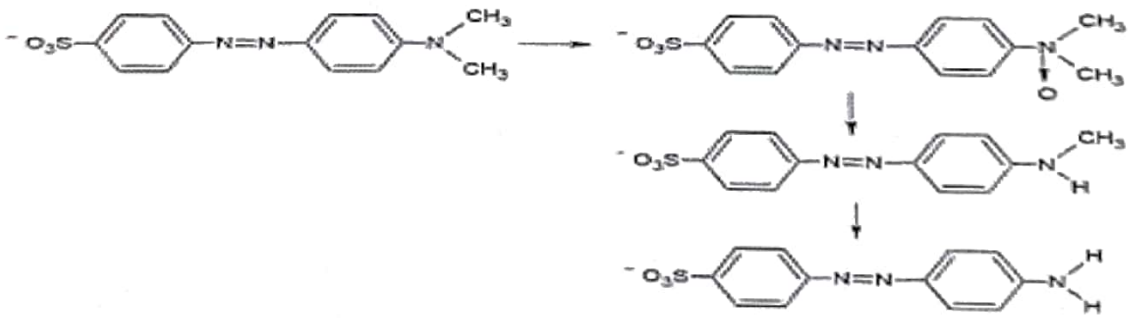


Figure 2. Proposed structures of different forms of methyl orange before and after bromination

Absorption spectra

Absorption spectra for determination of Clindamycin HCl, Clarithromycin and Cefixime were studied over range of 200 - 800 nm. After oxidation of the three drugs and portions of dyes with bromine, residual unoxidized methylene blue or methyl orange are absorbed at (666 nm (for clindamycin and cefixime) and (678 nm for clarithromycin) in case of methylene blue and 510 nm in case of methyl orange. (Fig. 2 and 3)

Effect of Acidity

Different acids were tested as a medium for bromine generation including sulphuric acid, hydrochloric acid, nitric acid and phosphoric acid. Hydrochloric acid produced the most precise and accurate results. Therefore, 5 M HCl was

ISSN 1110-5089

used throughout experiments. The best volume of HCl shown in tables 1and 2.

Effect of bromate - bromide volume

Bromate - bromide volume was studied by varying the reagent volume while other factors were held constant. It was found that 1 ml of bromine(except 0.8 ml for clarithromycin with methylene blue) is sufficient for its bleaching action using these stated concentrations (25, 12.5 µg/ml for methylene blue and methyl orange , respectively) tables 1and 2.

Effect of time

Time required to brominates and oxidize the drug before addition of dye and time required to irreversibly oxidize dye after its addition was studied. The bromination reaction was found to be complete in 10 minutes for clarithromycin and cefixime and (5 minutes for clindamycin HCl only with methyl orange), while contact times up to 25 minutes had been examined and no further bromination was detected. A contact time of 10 minutes (in case of methylene blue or 2 minutes (in case of methyl orange) for the bleaching of the dye colour by the residual bromine and the colour of the two dyes remains stable for at least two hours after mixing with the reaction mixture. Resultes are shown in tables (1), (2).

Effect of Dye volume

Dye volume was studied by varying the dye volume while other factors were held constant (Tables 1, 2)

Method validation

The developed methods were validated according to international conference on harmonization guidelines (Basavaiah and Tharpa, 2008). Calibration curves have correlation coefficients (r) higher than 0.999 indicating good linearity. The accuracy of the methods was determined by investigating the recovery of drugs at concentration levels covering the specified range (three replicates of each concentration). The results showed excellent recoveries tables (3), (4). Also, the Limit of detection (L.D.), Limit of quantitation (L.Q.), Sandell's sensitivity (S.S.) and Molar absorbitivity were calculated. Intraday precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drug table(8),(9). For interday reproducibility on a day - to - day basis, a series was run, in which the standard drug solutions were analyzed each for five days. The day - to - day SD values were shown in table (8) ,(9). The robustness of the methods was evaluated by making small changes in the volume of acid, dye volume and bromated bromine volume where the effect of the changes was studied on the percent recovery of drugs (Tables 10, 11).

Applications

Some Pharmaceutical formulations containing stated drugs have been successfully analyzed by the proposed methods. Results obtained were compared to those obtained by applying reported reference methods (El-Yazbi F et al., 1993; Suraga et al., 2008; Abdellatef et al., 2002) where Student's t-test and F-test were performed for comparison. The reported reference method of clarithromycin depend on formation of yellow colored chloroform extractable ion-association complexes of clarithromycin with bromothymol blue (BTB) and cresol red (CR) in buffered aqueous solution at pH 4. The extracted complexes showed maximum absorbance at 410 and 415 nm for BTB and CR, respectively .The reported reference method of clindamycin depend on oxidation of the sulfur atom in this drug with potassium iodate in acidic medium with the liberation of iodine and subsequent extraction with cyclohexane followed by measuring the absorbance at 520 nm. The reported reference method for cefixime is based on the alkaline hydrolysis of the drug and subsequent reactions of the resulting hydrolysates with NBD-Cl as a chromogenic reagent and detection at 401nm. Results are shown in tables 5, 6 and 7 where the calculated t and F values were less than tabulated values which in turn indicate that there is no

ISSN 1110-5089

significant difference between proposed methods and reference ones relative to accuracy and precision.

Conclusion

Unlike GC and HPLC techniques, spectrophotometry is simple and inexpensive. The proposed methods require only bromated-bromide mixture and dyes as reagents which are cheaper and readily available, no pH adjustment is required and the procedures do not involve any critical reaction conditions or tedious sample preparation. Morever, methods are simple, fast, accurate and adequately sensitive. The amounts obtained by the proposed methods are between 99.94% and 100.54%, within the acceptance level of 95% to 105%. The present methods are superior to the reference method with respect to both sensitivity and selectivity. The methods have been successfully applied for the analysis of marketed tablets and capsules.

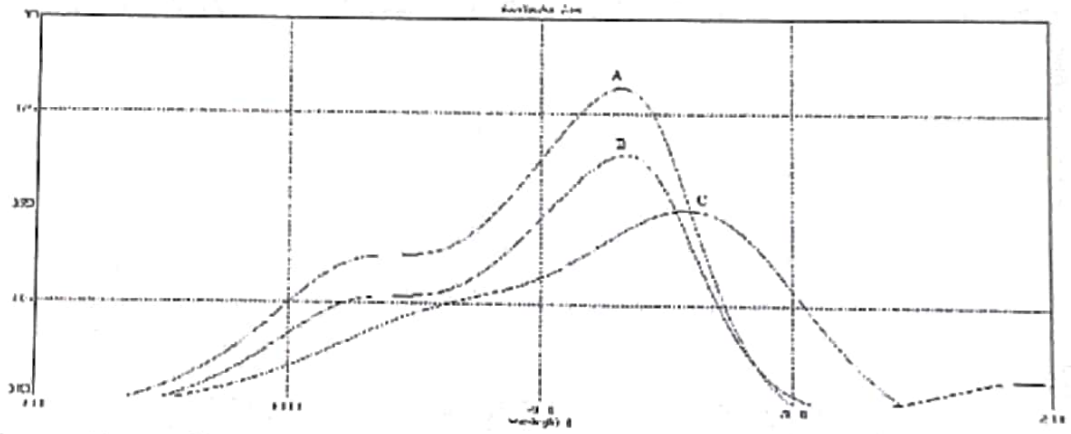


Figure 3. Absorption spectra of 60µg/ml methylene blue and 4 µg/ml ClindamycinHCl (A), Cefixime (B) and Clarithromycin (C) after bromine oxidation at 666 nm (efixime and Clindamycin) or 678 nm(Clarithromycin).

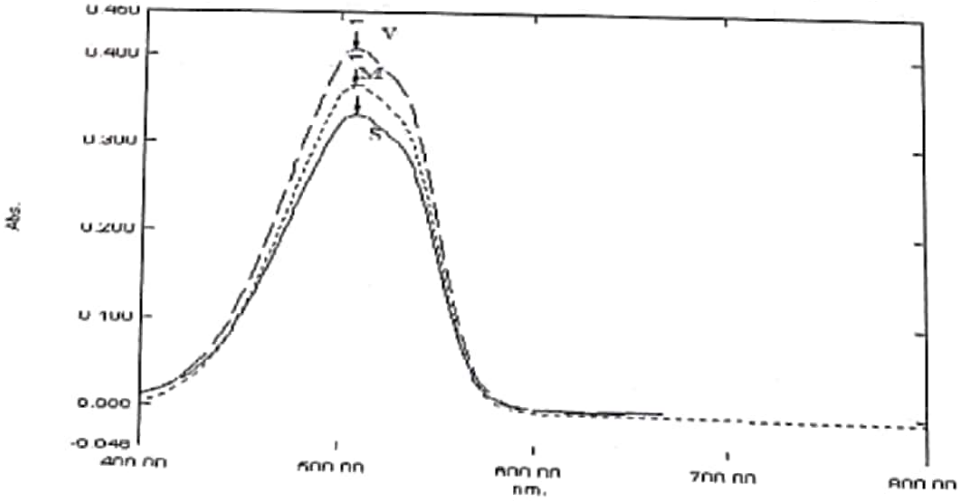


Figure 4. Absorption spectra of 60µg/ml methyl orange and 1 µg/ml of (Clindamycin HCl (M) and Cefixime (V) or 7 µg/ml of Clarithromycin (S) after bromine oxidation at 510 nm.

Table (1). Analytical parameters for the determination of Cefixime, Clarithromycin and Clindamycin HCl using methylene blue method.

Parameters	Methylene Blue (60µg/ml)		
	ClindamycinHCl	Clarithromycin	Cefixime
Wave length, nm	666	678	666
Volume of dye, ml	1	1	1
Volume of 5M HCl, ml	0.2	0.2	0.8
Volume of Bromate - Bromide mixture (25µg/ml) , ml	1	0.8	1
Time before dye addition, min	10	10	10
Time after dye addition, min	10	10	10
Beer's law limits, ug/ml	1.6-4.8	3.2-16	0.8-7.2
Regression equation	y=0.156x+0.088	y=0.036x+0.126	y=0.097x+0.171
Correlation Coefficient	0.999	0.999	0.999

y = a + bx, where **y** is the absorbance, **a** is the intercept, **b** is the slope and **x** is the concentration in ug/ml.

Table (2). Analytical parameters for the determination of Cefixime, Clarithromycin and Clindamycin HCl using methyl orange method.

Parameters	Methyl Orange (60µg/ml)		
	Clindamycin	Clarithromycin	Cefixime
Wave length, nm	510	510	510
Volume of dye, ml	1	1	1
Volume of 5M HCL, ml	0.6	0.2	0.6
Volume of Bromate - Bromide mixture (12.5µg/ml) , ml	1	1	1
Time before dye addition, min	5	10	10
Time after dye addition, min	2	2	2
Beer's law limits, µg/ml	0.8-4	6.4-19.2	0.4-2
Regression equation	y=0.171x+0.028	y=0.029x+0.073	y=0.276x+0.15
Correlation Coefficient	0.999	0.999	0.999

y = a + bx, where **y** is the absorbance, **a** is the intercept, **b** is the slope and **x** is the concentration in µg/ml.

Table (3). Results of the analysis for determination of Cefixime, Clarithromycin and Clindamycin HCl using methylene blue method.

Parameters	Methylene Blue								
	Clindamycin HCl *			Clarithromycin *			Cefixime*		
	Taken µg/ml	Found µg/ml	Recovery* %	Taken µg/ml	Found µg/ml	Recovery* %	Taken µg/ml	Found µg/ml	Recovery* %
	1.6	1.61	100.71	3,2	3.16	98.95	0.8	0.78	98.21
	2.4	2.43	100.31	6.4	6.38	99.82	2.4	2.42	101.19
	3.2	3.19	99.72	9,6	9.77	101.85	4	4.05	101.27
	4	3.98	99.68	12,8	12.86	100.47	5.6	5.63	100.58
	4.8	4.82	100.45	16	15.94	99.65	7.2	7.2	100.34
Mean			100.17			100.15			100.32
±SD			0.45			1.09			1.24
±RSD			0.45			1.09			1.23
±SE			0.204			0.488			0.55
Variance			0.209			1.19			1.54
Slope			0.156			0.036			0.097
L.D.			0.533			1.066			0.266
L.Q.			1.6			3.2			0.8
S.S.			0.005			0.011			0.0062
Apparent Molar absorbitivity $L.Mol^{-1}.cm^{-1}$			$7.99x10^6$			$4.04x10^7$			$7.41x10^7$

* Average of three independent procedures.

Zagazig J. Pharm. Sci. Dec., 2013 ISSN 1110-5089
Vol. 22, Issue. 2, pp, 58-68

Table (4). Results of the analysis for determination of Cefixime, Clarithromycin and Clindamycin HCl using methyl orange method.

Parameters	Methyl Orange								
	Clindamycin HCl *			Clarithromycin *			Cefixime*		
	Taken µg/ml	Found µg/ml	Recovery* %	Taken µg/ml	Found µg/ml	Recovery* %	Taken µg/ml	Found µg/ml	Recovery* %
	0.8	0.79	99.85	6,4	6.44	100.75	0.4	0. 397	99.45
	1,6	1.6	100.59	9,6	9.51	99.13	0.8	0.381	99.90
	2,4	2.43	101.57	12,8	13	101.56	1.2	1.21	101.27
	3,2	3.21	100.4	16	15.87	99.13	1.6	1.6	100.36
	4	4.01	100.29	19,2	19.31	100.57	2	1.99	99.81
Mean			100.54			100.23			100,16
±SD			0.63			1.06			0.702
±RSD			0.63			1.06			0.700
±SE			0.285			0.47			0.313
Variance			0.407			1.13			0.492
Slope			0.171			0.029			0.276
L.D.			0.266			2.133			0.133
L.Q.			0.8			6.4			0.4
S.S.			0.005			0.017			0.002
Apparent Molar absorbitivity $L.Mol^{-1}.cm^{-1}$			$8.02x10^6$			$2.66x10^7$			$1.99x10^6$

* Average of three independent procedures.

Table (5). Statistical analysis of results obtained by the proposed methods applied on Clindamycin in the Clindam tablets compared with reference method.

Parameters	Methylene Blue method	Methyl Orange method	Reported method
N	5	5	5
Mean	100.64	100.56	99.84
S D	0.264	0.357	1.226
RSD	0.514	0.598	1.226
SE	0.510	0.594	0.550
Variance	0.229	0.267	1.051
Student-t	1.423 (2.57)[a]	0.903 (2.57)[a]	
F-test	4.58(6.256)[b]	3.93(6.256)[b]	

a and b are the Theoretical Student t-values and F-ratios at p=0.05.

Table (6). Statistical analysis of results obtained by the proposed methods applied on Claritromycin in the Clarithro® tablets compared with reference method.

Parameters	Methylene Blue method	Methyl Orange method	Reported method
N	5	5	5
Mean	100.47	100.56	100.01
S D	0.612	0.902	1.353
RSD	0.782	0.949	1.353
SE	0.778	0.944	0.605
Variance	0.349	0.424	1.210
Student-t	0.692 (2.57)[a]	0.756 (2.57)[a]	
F-test	3.467 (6.256)[b]	2.853 (6.256)[b]	

a and b are the Theoretical Student t-values and F-ratios at p=0.05.

Table (7). Statistical analysis of results obtained by the proposed methods applied on Cefixime in the Ximacef® capsule compared with reference method.

Parameters	Methylene Blue method	Methyl Orange method	Reported method
N	5	5	5
Mean	100.16	100.42	99.99
S D	0.541	0.449	0.851
RSD	0.736	0.671	0.851
SE	0.735	0.667	0.383
Variance	0.329	0.229	0.735
Student-t	0.377 (2.57)[a]	1.002 (2.57)[a]	
F-test	2.234 (6.256)[b]	3.209 (6.256)[b]	

a and b are the Theoretical Student t-values and F-ratios at p=0.05.

Table (8). Results of the intraday and interday precision for the determination of Cefixime, Clarithromycin and Clindamycin HCl using methylene blue method.

Drug	conc. $\mu g.ml^{-1}$	Methylene blue			
		Intraday		Interday	
		Mean±SD	RSD	Mean±SD	RSD
Clindamycin	4	101.8 ± 0.40	0.4	100.4 ± 0.6	0.6
Clarithromycin	4	100.6 ± 0.74	0.74	100.06 ± 0.52	0.52
Cefixime	4	101.3 ± 0.76	0.76	99.9 ± 0.38	0.38

Table (9). Results of the intraday and interday precision for the determination of Cefixime, Clarithromycin and Clindamycin HCl using methyl orange method.

Drug	conc. $\mu g.ml^{-1}$	Methyl Orange			
		Intraday		Interday	
		Mean±SD	RSD	Mean±SD	RSD
Clindamycin	1	101.8 ± 0.86	0.86	101.3 ± 0.52	0.52
Clarithromycin	7	100.9 ± 0.27	0.27	100.9 ± 0.68	0.68
Cefixime	1	99.9 ± 0.68	0.68	100.4 ± 1.04	1.04

Table (10). Results of the robustness for the determination of Cefixime, Clarithromycin and Clindamycin HCl using methylene blue method.

Parameters	Methylene Blue		
	% of recovery ± SD		
	Clindamycin	Clarithromycin	Cefixime
HCl 0.18	100.9 ± 0.71	98 ± 1.9	99.2 ±0.81
HCl 0.22	100.9 ± 0.86	98.5 ± 1.4	100.6 ± 1.2
vol. of Br/Bro_3 0.95	100.8 ±0.35	98.1 ±1.5	99±0.69
vol. of Br/Bro_3 0.95	98.03 ± 1.2	99 ± 0.69	98.9± 0.96
dye 0.95	101.7 ± 1.01	98..14 ± 0.19	99.05 ± 0.69
dye 1.05	99.8 ± 0.66	101.3 ± 0.88	98.6 ± 1.06

Table (11). Results of the robustness for the determination of Cefixime, Clarithromycin and Clindamycin HCl using methy orange method.

Parameters	Methyl Orange		
	% of recovery ± SD		
	Clindamycin	Clarithromycin	Cefixime
HCl 0.18	98.5 ± 1.1	99.7 ± 0.53	98.7 ± 1.3
HCl 0.22	98.3 ± 1.6	101.8± 0.71	101.7 ± 1.18
vol. of Br/Bro_3 0.95	98.7 ±1.3	101.9 ±0.53	100.4±0.71
vol. of Br/Bro_3 0.95	101.8 ±0.51	101.92 ± 0.77	100± 0.92
dye 0.95	100.8 ± 0.46	99.6 ± 0.80	98.03± 1.4
dye 1.05	101.8 ± 0.51	101.9 ± 1.09	98.2 ± 1.04

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استخدام البرومين في تعيين كل من السيفيكزيم , الكلاريثروميسن والكليندااميسن في صورهم النقية ومستحضراتهم الصيدلية

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يصف هذا الجزء طريقتين لتحليل كل من السيفيكزيم , الكلاريثروميسن والكليندااميسن في صورهم النقية ومستحضراتهم الصيدلية. وتعتمد الطريقتين على الانتاج اللحظى للبرومين كعامل مؤكسد واستخدام اما الميثلين الازرق او الميثيل البرتقالى ككاشف طيفى. فتتم أكسدة تلك الادوية باستخدام البرومين المنتج لحظيا حيث تستهلك جزء من ذلك العامل المؤكسد والجزء المتبقى يؤكسد جزء من الكاشف (الميثلين الازرقا او الميثيل البرتقالى) تاركا جزءاً اخر يتم قياسه طيفياً عند طول موجى (666 السيفيكزيم والكليندااميسن و678 الكلاريثروميسن) او (للادوية الثلاثة 510)نانومتر على التوالى حيث ان كمية البرومين الداخلة فى التفاعل تتناسب مع تركيز الدواء المؤكسد. وقد تمت دراسة العوامل المختلفة التى تؤثر على التفاعل كالحامضية، تركيز العامل المؤكسد والوقت. وقد أُتبع قانون بيير على مدى تركيز قدره (3.2-16) ميكروجرام/ملليلتر لمادة الكلاريثروميسن و (1.6-4.8) ميكروجرام/ملليلتر لمادة الكليندااميسن و(0.8-7.2) لمادة السيفيكزيم فى حالة الميثلين الازرق، على مدى تركيز قدرة (6.4-19.2) ميكروجرام/ملليلتر لمادة الكلاريثروميسن و(0.8-4)ميكروجرام/ملليلتر لمادة الكليندااميسن و(0.4-2)لمادة السيفيكزيم فى حالة الميثيل البرتقالى. وقد أُستخدمت الطرق فى تعيين هذه الادوية فى بعض مستحضراتهم الصيدلية وتمت مقارنة النتائج إحصائياً مع الطرق المرجعية.