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Application of ion-pair complexation reaction for the spectrophotometric determination of clarithromycin in pharmaceutical formulations

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ABSTRACT

Objective: To develop two simple, rapid, sensitive, selective and extraction-free spectrophotometric methods for the determination of clarithromycin (CLR) both in pure form and in pharmaceutical formulations. Methods: The developed methods are based on ion-pair complexation reaction between CLR and two sulphonphthalein acid dyes, bromothymol blue (BTB) and bromocresol purple (BCP). The yellow colored products were measured spectrophotometrically at 416 nm and 418 nm for BTB and BCP, respectively. Results: Beer's law is obeyed over the ranges of 0.5–25.0 μg/ml and 1.0–25.0 μg/ml, for BTB method and BCP method, respectively. The apparent molar absorptivities and Sandell's sensitivities (in L mol–1 cm–1 and μg cm–2 per 0.001 absorbance unit, respectively) were 2.01 × 104 and 0.0373 for BTB method, and 2.52 × 104 and 0.0297 for BCP method. The limits of detection (LOD) and quantification (LOQ) for both the methods are calculated. Conclusion: The developed methods were successfully applied to the determination of CLR in commercial tablets with good accuracy and precision and without detectable interference from common excipients. The accuracy and reliability of the developed methods were further established by parallel determination by the reference method and also by recovery studies via standard addition technique

INTRODUCTION

Clarithromycin (CLR), chemically known as 6-O-methylerythromycin (Figure 1). It has the empirical formula $C_{38}H_{69}NO_{13}$ with a molecular weight of 748.0 g/mole [1]. It is a semi-synthetic macrolide antibiotic with a broad antibacterial spectrum and it has a good stability in gastric acid and a more favorable pharmacokinetic profile than erythromycin [2].

A literature survey revealed that several methods to determine CLR in pharmaceutical formulations have been reported. These include different techniques such as high performance liquid chromatography [3-9], ultraviolet (UV) spectrophotometry [10,11], visible spectrophotometry [12-37], and voltammetry [38,39]. Many of these methods suffered from one or other disadvantage such as use of non-selective reagents, poor sensitivity, slow reaction, heating or extraction step, narrow linear dynamic range, use of expensive chemicals, and/or complicated experimental setup as explained in Table 1.

This work describes the application of ion-pair complexation reaction for the visible spectrophotometric determination of CLR in pure drug as well as in tablets. The developed methods are based on the formation of yellow colored ion-pair complexes between CLR and two sulfonphthalein acid dyes, namely, bromothymol blue (BTB) and bromocresol purple (BCP) with absorption maximum at 416 and 418 nm for BTB and BCP, respectively. The formed ion-pair complexes in both of the developed methods require no extraction step and measured directly in chloroform, confirming the simplicity of the developed methods.

EXPERIMENTAL

Apparatus

A UV-visible spectrophotometer model 752 (Ningbo Hinotek Technology Co. Ltd., Zhejiang, China) was used for all absorbance measurements.

Materials and Methods

All reagents and solvents used were of analytical reagent grade.

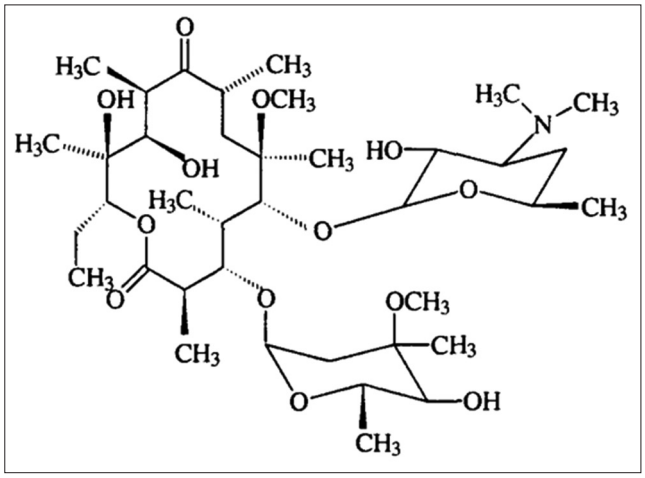


Figure 1: Chemical structure of clarithromycin

Solutions of 0.2% w/v BTB and 0.2% w/v BCP were prepared in chloroform.

Standard CLR solution: A stock standard CLR solution (100 μg/ml) was prepared by dissolving of 10 mg of pure CLR in chloroform in a 100 ml standard flask and diluting to the volume with chloroform. The working standard CLR solution (50 μg/ml) was then prepared by a suitable dilution of the stock standard solution with chloroform.

The pharmaceutical preparation used in this study was claromin-250 mg/tablet which is marketed by Modern Pharma Company, Sana'a, Yemen.

Procedures

Preparation of calibration curves

BTB method (using BTB)
Different aliquots (0.1, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 ml) of a standard solution (50 µg/ml of CLR) were accurately transferred into a series of 10 ml calibrated flasks using a micro burette. To each flask was added 1 ml of 0.2% BTB solution and after 5 min, the mixture was diluted to the mark with chloroform and mixed well. The absorbance of each solution was measured at 416 nm against a blank.

BCP method (using BCP)
Different aliquots (0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 ml) of a standard CLR (50 µg/ml) solution were accurately transferred into a series of 10 ml calibrated flasks. To each flask, 1 ml of 0.2% BCP solution was added. After 5 min, the mixture was diluted

Table 1: Comparison of performance characteristics of the developed methods with the existing visible spectrophotometric methods

Reagent/s used	λ_{max} nm	Linear range, µg/ml and ε (L mol^{-1} cm^{-1})	LOQ μg/ml	Remarks	References
a) BTB	410	0.1-20 $\varepsilon=2.01\times10^4$	0.1	Extraction step is required	[12]
b) Cresol red	415	2.0-20 $\varepsilon=4.378\times10^3$	2.0		
P-dimethylamino benzaldehyde	600	10-70 $\varepsilon=6.8313\times10^3$	-	Less sensitive and use of concentrated H_2SO_4, narrow linear range	[13]
a) O-nitro benzaldehyde	480	0.5-3.0 $\varepsilon=1.063\times10^5$	-	Use of concentrated HCl and slow reaction in method (a), heating step is required for methods b and c	[14]
b) Fe (III)	480	0.5-3.0 $\varepsilon=1.032\times10^5$	-		
c) DDQ	450	5-30 $\varepsilon=4.951\times10^3$	-		
Alizarin	546	1-100 $\varepsilon=7.31\times10^3$	-	Less sensitive	[15]
Chloranilic acid	491	8-40 $\varepsilon=1.67\times10^4$	1.12	Narrow linear dynamic range	[16]
Haematoxylin reagent/Boric acid	587	0.2-4.0 $\varepsilon=1.552\times10^5$	0.142	Slow reaction (stand for 30 min)	[17]
DDQ/microwell assay	450	20-850	51.2	Less sensitive	[18]
$FeCl_3$/KSCN	445	2-22	1.75	heating step is required, slow reaction (60 min)	[19]

(*Contd...*)

Table 1: (*Continued*)

Reagent/s used	λ_{max} nm	Linear range, μg/ml and ε (L mol^{-1} cm^{-1})	LOQ μg/ml	Remarks	References
Thymol blue/ethanol	442	8.278-57.59 ε=1.66×10^4	-	Narrow linear dynamic range	[20]
Purpurin/alcohol	548	10-150 ε=4.49×10^3	-	Less sensitive	[21]
a) Fe (III)/potassium ferricyanide	740	12.5-75.0 ε=1.166×10^4	-	Slow reactions	[22]
b) Folin-Ciocalteu	775	25-125 ε=4.368×10^3	-		
$FeCl_3$/1,10-phenanthroline	515	0.05-0.25	-	Narrow linear dynamic range	[23]
Tropaeolin	500	10-40 ε=1.975×10^3	-	Less sensitive, narrow range	[24]
Marquis	495	10-70 ε=7.5045×10^3	-	Narrow linear dynamic range	[25]
2-nitrobenzaldehyde	486	10-45	-	Slow reaction	[26]
Rose Bengal/copper	557	7.5-40 ε=1.47×10^4	2.8	Extraction step is required	[27]
Eosin Y	542-544	3-30 ε=4.367×10^4	0.906	Narrow linear dynamic range	[28]
BCG	415	-	-	Extraction step is required	[29]
2,4-dinitrophenylhydrazine	523-526	5-35	-	Narrow linear dynamic range	[30]
$KBrO_3$ – KBr					
a) Methylene blue	678	3.2-16 ε=4.04×10^7	3.2	Non-selective	[31]
b) Methyl orange	510	6.4-19.2 ε=2.66×10^7	6.4		
a) BCP	390	12-28 ε=1.31×10^7	7.95	Extraction step is required	[32]
b) BCG	413	4-20 ε=1.1×10^5	15.8		
a) Concentrated HCl/acetone	485	50-500	-	Extraction step is required in all three methods	[33]
b) BCG	414	0-60	-		
c) BPB	414	0-60	-		
HCl/Folin-Ciocalteu	760.5	20-120	-	Slow reaction	[34]
a) I_2	363	35-135 ε=2985–3497	-	Less sensitive	[35]
b) TCNE	420	15-95 ε=6877–9872	-		
Quinalizarin	580	0-100 ε=3.74×10^3	-	Less sensitive	[36]
BPB	414	10-40	-	Extraction step is required	[37]
a) BTB	416	0.5-25 ε=2.01×10^4	1.10	Simple, sensitive, extraction-free, only one reagent required	Present methods
b) BCP	418	1.0-25 ε=2.52×10^4	0.72		

DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, TCNE: tetracyanoethylene, BTB: Bromothymol blue, BCP: Bromocresol purple, LOQ: Limit of quantification, BCG: Bromocresol green, BPB: Bromophenol blue

to the mark with chloroform and mixed well. The absorbance of each solution was measured at 418 nm against a blank.

Procedure for tablets

The content of seven tablets of claromin-250 was weighed accurately and ground into fine powder in a mortar. A portion of the powder equivalent to 20 mg of the active component (CLR) was accurately weighed and transferred into a 100 ml calibrated flask, and dissolved in about 50 ml of chloroform. The solution was shaken thoroughly for 20 min, diluted to the volume with the same solvent and filtered using a Whatman No. 42 filter paper. The first portion of the filtrate was rejected and aliquots of this filtrate were subjected to analysis by the procedure described above after its dilution to 50 μg/ml in CLR with chloroform.

Procedures for method validation

Linearity, limits of detection (LOD) and quantification (LOQ)
Linearity was assessed by analyzing a set of seven calibration standards. Calibration curves for both methods were prepared as described under "Preparation of calibration curves." The linearity of both the methods was determined by the method of least squares and plotting of the absorbance (Y) against the concentration (X) of CLR. The LOD and LOQ values were calculated using the formulae [40]:

$$\text{LOD} = \frac{3.3S}{b} \text{ and } \text{LOQ} = \frac{10S}{b}$$

Where S is the standard deviation of seven replicates for the absorbance of blanks and b is the slope of the calibration curve.

Precision and accuracy
To evaluate the precision and accuracy of the developed methods, solutions containing three different concentrations of CLR were prepared and analyzed in five replicates on the same day (intra-day) and on 4 consecutive days (inter-day). Mean and standard deviations were obtained by back-calculated CLR concentration at each level. Precision and accuracy were calculated in terms of relative standard deviation (RSD) and relative error (RE), respectively.

Robustness and ruggedness
The robustness of the developed methods was examined by evaluating the influence of small incremental changes in two experimental variables, namely, volume of the dye and the reaction time, on the performance of the developed methods. The analysis was performed at the altered experimental conditions by taking three different concentrations of CLR and studying the effect of the changes on the absorbance reading of the formed ion-pair complexes in both the methods by calculating the %RSD each time. Method ruggedness of the developed methods was examined by performing the analysis by three different analysts and also by a single analyst using three different cuvettes. These assays were performed at three concentration levels of CLR.

Procedures for Selectivity Study

The effect of common excipients added to pharmaceuticals was examined by the developed methods for their possible interferences with the determination of CLR by placebo blank and synthetic mixture analyses. A placebo blank containing starch, talc, lactose, calcium gluconate, magnesium stearate, and sodium dihydrogen phosphate was extracted with chloroform and solutions were made as described under "Procedure for tablets." A suitable amount of solution was analysis by both the methods following the procedures described under "Preparation of calibration curves." 10 mg of pure CLR was added to the placebo blank of similar composition described above and mixed well. The solution of the synthetic mixture equivalent to 100 μg/ml of CLR was prepared as described under "Procedure for tablets." The filtrate was collected and 1.0 ml of the resulting solution was assayed (n = 5) by both the methods.

RESULTS AND DISCUSSION

Absorption Spectra

The reaction of BTB or BCP with CLR in chloroform medium results in the formation of yellow colored ion-pair complexes. The absorption spectra of the yellow colored products were recorded at 380-480 nm against the corresponding blank solutions and are shown in Figure 2. The yellow colored ion-pair complexes showed maximum absorbance at 416 and 418 nm for CLR-BTB and CLR-BCP, respectively.

Reaction Pathway

CLR contains tertiary amino group, nitrogen atom bonded to electron donating methyl groups, which can be protonated and formation of ion-pair complexes with dyes such as BTB and BCP. BTB and BCP are examples for dyes of sulfonphthalein type, and the color of such dyes is due to the opening of lactoid ring and subsequent formation of the quinoid group. It supposed the presence of the equilibrium between the two tautomers but due to strong acidic nature of the sulfonic acid group, the quinoid body must predominate [41]. Finally, the protonated CLR forms ion-pair complexes with the dyes. The possible reaction pathways are proposed and presented in Scheme 1.

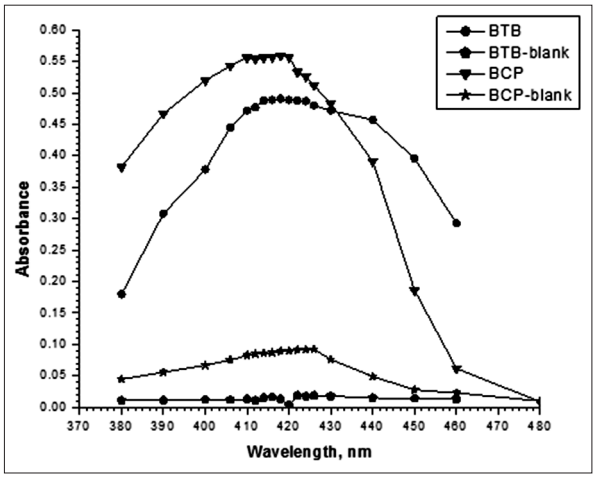
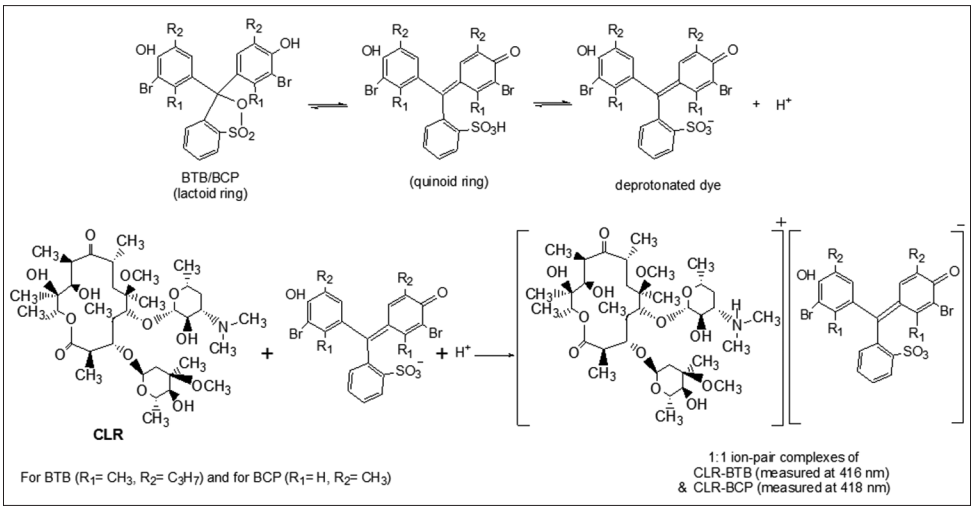


Figure 2: Absorption spectra of ion-pair complexes of: Clarithromycin (CLR)-bromothymol blue and CLR-bromocresol purple against reagent blank



Scheme 1: The possible reaction pathway for the formation of clarithromycin (CLR)-bromothymol blue and CLR-bromocresol purple ion-pair complexes

Optimization of Reaction Conditions

To study the effect of the dye concentration on the intensity of the yellow colored complexes, the absorbances of solutions containing a fixed concentration of CLR (12 µg/ml) and different amounts (0.25-2.5 ml) of the respective dyes of 0.2% BTB or 0.2% BCP solutions were measured. Maximum absorbances of both complexes were achieved with 1 ml of each BTB and BCP solution and excess dyes did not affect the absorbance of the complex. The formed ion-pair complexes were stable for at least 1 h in both the methods.

The Japanese Pharmacopoeia [42] reported that CLR is soluble in acetone and in chloroform. To select the suitable solvent for preparation of CLR and dyes solutions, CLR and both dyes were prepared separately in two solvents, namely, acetone and chloroform; the same solvent was used as diluting solvent. Then, the reaction of CLR with BTB or BCP was carried out in the solvents mentioned above and the absorbance of each solution was measured against the corresponding blank at the selected wavelengths. The results showed that the chloroform is a suitable solvent for ion-pair complex formation in both the methods because it gives the maximum sensitivity when compared with acetone.

The effect of the time on the reaction of CLR with BTB or BCP in chloroform medium was studied at different values (0-45 min) by continuous monitoring of the absorbance at the selected wavelengths. It was found that the reaction is complete and quantitative when the reaction mixture was allowed to stand for 5 min and any delay in the absorbance measurements of the formed complexes up to 1 h had no effect on the reaction stoichiometry which is determined to be 1:1 (CLR:dye) for the ranges studied.

Stoichiometric Relationship

To determine the stoichiometric ratio between CLR and BTB/BCP, Job's method of continuous variation was employed. In this method, solutions of 6.68×10^{-5} M standard CLR and 6.68×10^{-5} M dye (BTB/BCP) were mixed in varying volume ratios in such a way that the total volume of each mixture was kept the same. The absorbance of each solution was plotted against the mole fraction of the drug, $\frac{V_{CLR}}{V_{CLR}+V_{dye}}$ (Figure 3). As shown in Figure 3, the molar ratio which gave maximum absorbance was 0.5, indicating the formation of 1:1 (CLR:dye) ion-pair complexes and confirm the presence of one basic nitrogen-containing group in the drug. The formation constants (K_f) of the formed complexes were calculated from the data of continuous variations method using the following equation [43]:

$$K_f = \frac{A/A_m}{(1 - A/A_m)^{n+2} C_M (n)^n}$$

Where, A and A_m are the observed maximum absorbance and the absorbance value when all the drug present is associated, respectively. C_M is the molar concentration of drug at the maximum absorbance and n is the stoichiometry. The K_f values were found to be 1.37×10^7 and 1.42×10^7 for CLR-BTB and CLR-BCP complexes, respectively. The high values of K_f confirm the expected high stabilities of the formed ion-pair complexes.

Method Validation

The developed methods were validated according to the current ICH guidelines for validation of analytical procedures [40].

Linearity and sensitivity

A linear correlation between the absorbance of the formed ion-pair complexes CLR-BTB and CLR-BCP at the selected wavelengths, and the concentration of CLR was found in the ranges presented in Table 2. The linear regression equations for both the methods were obtained and the Beer's law range, correlation coefficient, molar absorptivity, Sandell's sensitivity, LOD, and LOQ values for both the methods are presented in Table 2. The linearity of the calibration curves was confirmed by the high values of correlation coefficients and low values of LOD and LOQ.

Precision and accuracy

The results of intra- and interday precision and accuracy for the developed methods are presented in Table 3. The low values of the percentage %RSD indicate the high precision, and the values of percentage %RE show the good accuracy of the developed methods.

Interference studies

Suitable aliquots of placebo blank solution prepared under "Procedures for selectivity study" was subjected to analysis by both the methods, and the results showed that the absorbance almost equal to that of the reagent blank confirming the absence of the interferences from the added excipients. A suitable volume of synthetic mixture solution prepared under "Procedures for selectivity study" was also subjected to analysis by both the methods and the percent recoveries of CLR were calculated and found to be 101.9 ± 2.03 and 99.64 ± 1.97 (n = 5) by BTB and BCP methods, respectively. The calculated percentage recoveries are good and revealed that no interference was observed from any of the added excipients in both of the developed methods.

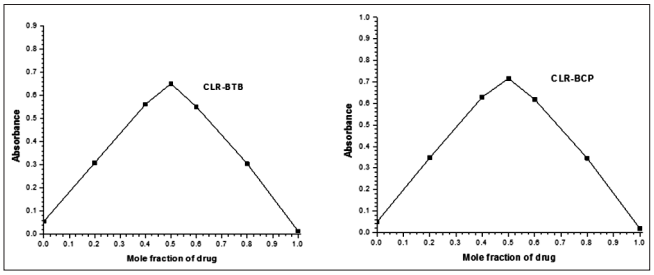


Figure 3: Job's continuous - variations plots

Table 2: Analytical parameters for the determination of CLR using the developed methods

Parameter	BTB method	BCP method
λ_{max}, nm	416	418
Beer's law limits, μg/ml	0.5-25.0	1.0-25.0
Molar absorptivity, L mol^{-1} cm^{-1}	2.01×10^4	2.52×10^4
Sandell sensitivity*, μg/cm^2	0.0373	0.0297
Limit of detection, μg/ml	0.36	0.24
Limit of quantification, μg/ml	1.10	0.72
Regression equation, Y**		
Intercept (a)	0.0053	0.0024
Slope (b)	0.0232	0.0322
Correlation coefficient (r)	0.9998	0.9997
Standard deviation of intercept (S_a)	0.00312	0.00474
Standard deviation of slope (S_b)	0.00022	0.00034
Variance (S_a^2)	9.73×10^{-6}	2.25×10^{-5}

*Limit of determination as the weight in μg per ml of solution, which corresponds to an absorbance of A=0.001 measured in a cuvette of cross-sectional area 1 cm^2 and l=1 cm. **Y=a+bX, where Y is the absorbance and X is the concentration in μg/ml

Robustness and ruggedness

The results of the robustness and ruggedness of the developed methods are shown in Table 4 and confirm that the developed methods were robust as well as rugged.

Applications to Analysis of Pharmaceutical Formulations

The developed methods were successfully applied for the determination of CLR in claromin-250 tablets. The results shown in Table 5 indicated that the developed methods are successful for the determination of CLR and the excipients in the dosage forms do not interfere. A statistical comparison of the results for the determination of CLR by the developed methods and British Pharmacopeia reference method [1] is shown in Table 5. Statistical analysis of the results using Student's t-test for accuracy and F-test for precision confirmed that no significant difference between the developed methods and reference method at the 95% confidence level with respect to accuracy and precision (Table 5).

The accuracy of the developed methods was further ascertained by performing recovery experiment using the standard addition procedure. The results of this study were presented in Table 6 and reveal that the various excipients present in the formulations did not interfere.

CONCLUSIONS

This article describes the application of ion-pair complexation reaction for the visible spectrophotometric determination of CLR in bulk drug as well as in tablets. The developed methods have the advantages of simplicity, sensitivity, extraction-free and wide linear dynamic range, over most of the previously reported visible spectrophotometric methods for the determination of CLR in pharmaceuticals as can be seen from Table 1. The

Table 3: Evaluation of the precision and accuracy for the analysis of CLR in pure form using the developed methods

Method	CLR taken (μg/ml)	Intra-day (n=5)			Inter-day (n=4)		
		CLR found[a] (μg/ml)	%RSD[b]	%RE[c]	CLR found[a] (μg/ml)	%RSD[b]	%RE[c]
BTB method	5.00	5.08	0.97	1.60	5.10	1.86	2.00
	10.00	10.12	1.23	1.20	9.77	2.07	2.30
	15.00	15.31	1.45	2.07	14.66	1.98	2.27
BCP method	5.00	5.05	1.56	1.00	5.06	1.77	1.20
	10.00	9.73	1.29	2.70	9.72	2.14	2.80
	15.00	14.67	2.25	2.20	15.40	2.30	2.67

[a]Mean value of n determinations, [b]Relative standard deviation (%), [c]Bias (%): [(found – taken)/taken] ×100. CLR: Clarithromycin, BTB: Bromothymol blue, BCP: Bromocresol purple, RSD: Relative standard deviation, RE: Relative error

Table 4: Robustness and ruggedness of the developed methods.

Method	CLR taken (μg/ml)	Robustness (% RSD)		Ruggedness (%RSD)	
		Volume of the dye*	Reaction time*	Inter-analysts (n=3)	Inter-instruments (n=3)
BTB method	5.00	1.32	1.12	0.91	1.84
	10.00	1.05	0.93	0.89	2.12
	15.00	1.82	1.20	1.03	2.75
BCP method	5.00	1.00	1.31	1.02	1.92
	10.00	0.97	1.48	1.35	2.64
	15.00	1.66	2.02	1.42	2.81

*In both the methods, the volume of the dye was 0.9, 1.0 and 1.1 ml and the reaction time was 4, 5 and 6 min. CLR: Clarithromycin, BTB: Bromothymol blue, BCP: Bromocresol purple, RSD: Relative standard deviation

Table 5: Results of assay of claromin-250 tablets and statistical evaluation

Tablet brand name	Nominal amount, mg	Found % (of nominal amount±SD)*		
		Reference method	Proposed methods	
			BTB method	BCP method
Claromin	250	99.65±1.02	98.75±1.06	99.11±2.01
			t=1.37	t=0.56
			F=1.08	F=3.88

*Mean value of five determinations. Tabulated t-value at the 95% confidence level is 2.78; tabulated F-value at the 95% confidence level is 6.39. BTB: Bromothymol blue, BCP: Bromocresol purple, SD: Standard deviation

Table 6: Results of spike-recovery studies from pre-analyzed tablets

Method	Tablet studied	CLR in tablet, μg/ml	Pure CLR added, μg/ml	Total found, μg/ml	Pure CLR recovered[a], (percent±SD)
BTB method	Claromin (250 mg)	5.92	3.00	8.96	101.33±1.05
		5.92	6.00	12.07	102.50±1.37
		5.92	9.00	15.00	100.89±1.18
BCP method	Claromin (250 mg)	5.95	3.00	8.93	99.33±0.88
		5.95	6.00	11.99	100.67±1.24
		5.95	9.00	15.04	101.00±2.11

[a]Mean value of three determinations. BTB: Bromothymol blue, BCP: Bromocresol purple, SD: Standard deviation, CLR: Clarithromycin

developed methods are based on the simple mixing of CLR solution and dye solution in chloroform medium and measuring the absorbance. The statistical parameters and the recovery data reveal good accuracy and precision of both the methods. Moreover, the developed methods can be performed at room temperature and are suitable for application in laboratories when chromatographic techniques are not available.

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REFERENCES

1. HMSO: British Pharmacopoeia. Vol. I and II. London: Her Majesty's Stationery Office; 2009.
2. Amini H, Ahmadiani A. Sensitive determination of clarithromycin in human plasma by high-performance liquid chromatography with spectrophotometric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2005;817:193-7.
3. Srinivasu T, Rao BN, Mathrusri A, Ashutosh S, Chandrashekhar TG. Development and validation of high performance liquid chromatography method for quantification of related substances in clarithromycin powder for an oral suspension dosage form. Int J Anal Pharm Biomed Sci 2012;1:1-12.
4. Li P, Xiong AZ, Kuang P. Determination of clarithromycin in capsule by HPLC. Chin J Antibiot 2002;27:502-3.
5. Prasad Bathula SNV, Seshagiri Rao JVLN. A simple and sensitive liquid chromatography–mass spectrometry routine method for determination of clarithromycin in human plasma: Application to a clinical pharmacokinetic study. Clin Res Regul Aff 2010;27:60-6.
6. Gangishetty S, Verma S. RP-HPLC method development and validation for simultaneous estimation of clarithromycin and paracetamol. ISRN Anal Chem 2013;2013:948547.
7. Morgan DK, Brown DM, Rotsch TD, Plasz AC. A reversed-phase high-performance liquid chromatographic method for the determination and identification of clarithromycin as the drug

substance and in various dosage forms. J Pharm Biomed Anal 1991;9:261-9.
8. Koteswara Rao B, Manjula KR, Nageswara Rao M, Babu KS, Rambabu C. A new stability indicating RP-HPLC method for the simultaneous estimation of gemcitabine and clarithromycin in tarceva tablets. Indo Am J Pharm Res 2015;5:1613-24.
9. JieMing S. Determination of the content of clarithromycin by HPLC with gradient elution. Pract Pharm Clin Remed 2009;12:418-9.
10. Tripathi GK, Singh S, Pandey BV. UV spectroscopy technique for analysis to clarithromycin for gastric pH sensitive formulation. J Chem Pharm Res 2013;5:1258-62.
11. Rajesh KP, Somashekar S, Mallikarjuna GM, Kumar SMS. A sensitive UV spectrophotometric analytical method development, validation and preformulation studies of clarithromycin. Res J Pharm Tech 2011;4:242-6.
12. Shah J, Rasul Jan M, Manzoo S. Extractive spectrophotometric methods for determination of clarithromycin in pharmaceutical formulations using bromothymol blue and cresol red. J Chin Chem Soc 2008;55:1107-12.
13. Srinivasa Rao Y, Chowdary KPR, Seshagiriao JVLN. A new spectrophotometric method for determination of Clarithromycin tablets. Int J Chem Soc 2003;1:225-226.
14. Mruthyunjaya Rao R, Naresh S, Pendem K, Ramachandra Rao PSNH, Sastry CSP. Simple spectrophotometric methods for determination of clarithromycin in pure state and tablets. Asian J Chem 2012;24:1535-7.
15. Huakan L, Qiaofeng W, Yan Z. Spectrophotometric determination of clarithromycin based on charge transfer reaction between clarithromycin and alizarin. J Fourth Military Med Univ 2004;23:2206-8.
16. Sultana N, Arayne MS, Ali SN. The use of chloranilic acid for the spectrophotometric determination of three macrolides through charge transfer complex. Med Chem 2013;3:241-6.
17. Gouda AA, El-Sheikh R, Khalil KM. Spectrophotometric determination of certain macrolide antibiotics in pharmaceutical preparations. Int J TechnoChem Res 2015;1:11-24.
18. Darwish IA, Alqarni MA, Wani TA. Novel microwell assay with high throughput and minimum consumption for organic solvents in the charge transfer-based spectrophotometric determination of clarithromycin in pharmaceutical formulations. Chem Cent J 2013;7:172.
19. Khodavirdilo B, Khodavirdilo S, Najafi K. Simple spectrophotometric method for measuring antibiotics by using $Fe(SCN)^{2+}$ complex pharmaceutical products. Recent Adv Pharm Sci Res 2013;2:103-9.
20. Jun L, Yan L, Guo-Qing W, Na W, Quan-Min L. Spectrophotometric determination of clarithromycin by charge-transfer reaction between clarithromycin and thymol blue. Chem Res Appl 2012;4:565-8.
21. Li HK, Xiao JK. Spectrophotometric determination of clarithromycin based on the charge transfer reaction between clarithromycin and purpurin. Chin J Anal Chem 2005;33:1327-9.
22. Seshagiri Rao JVLN, Srinivasa Rao Y, Murthy TK, Sankar DG. Spectrophotometric estimation of clarithromycin in pharmaceutical formulations. Asian J Chem 2002;14:647-50.
23. Rao YS, Murthy TK, Chowdary KPR, Seshagiri Rao JVLN. Assay of clarithromycin in pharmaceutical formulations. Indian Drugs 2002;39:348-9.
24. Sudheer P, Ganapathi PAV. Extractive spectrophotometric estimation of clarithromycin in pharmaceutical formulations. Indian Drugs 2001;38:358-60.
25. Srinivasa Rao Y, Rajani Kumar V, Seshagiri Rao JVLN. Visible spectrophotometric determination of clarithromycin in pharmaceutical solid dosage forms. Asian J Chem 2002;14:1791-3.
26. Arbad BR, Jadhav SM. A simple spectrophotometric method for the determination of clarithromycin from pharmaceutical preparation. Asian J Chem 1999;11:1074-6.
27. Sayed RA, Hassan WS, El-Mammli MY, Shalaby A. New spectrophotometric and conductometric methods for macrolide antibiotics determination in pure and pharmaceutical dosage forms using Rose Bengal. J Spectrosc 2013;2013:214270.
28. Walash MI, Rizk MS, Eid MI, Fathy ME. Spectrophotometric determination of four macrolide antibiotics in pharmaceutical formulations and biological fluids via binary complex formation with eosin [corrected]. J AOAC Int 2007;90:1579-87.
29. Srinivasa Rao Y, Jitendrababu V, Chowdary KPR, Seshagiri Rao JVLN. Extractive spectrophotometric method for the determination of clarithromycin. Indian J Pharm Sci 2003;65:653-5.
30. Abdelmageed OH. Development and validation of a spectrophotometric method for the determination of macrolide antibiotics by using 2,4-dinitrophenylhydrazine. J AOAC Int 2007;90:364-71.
31. El-Adl SM, El-Sadek ME, Hassan MH. Bromometric estimation of cefixime, clarithromycin and clindamycin in bulk and dosage forms. Asian J Pharm Anal 2014;4:137-46.
32. El-Adl SM, El-Sadek ME, Hassan MH. Extractive spectro estimation of clarithromycin and clindamycin in bulk and dosage forms. Asian J Res Pharm Sci 2014;4:179-86.
33. Singhvi I. Visible spectophotometric methods for estimation of clarithromycin from tablet formulation. Indian J Pharm Sci 2002;64:480-2.
34. Nangude S, Vite M, Chugh N. Development of UV spectrophotometric method for estimation of clarithromycin in pharmaceutical dosage forms by using Folin-Ciocalteu reagent. Int J Pharm Chem Sci 2013;2:640-2.
35. El-Zaria ME, Etaiw SH. Two spectrophotometric methods for estimation of clarithromycin in pharmaceutical formulations and human plasma based on charge transfer complexes. Can J Anal Sci Spectrosc 2007;52:316-24.
36. Hua-Kan L, Yue L, Yu-Hua W. The spectrophotometric determination of clarithromycin based on the charge transfer reaction between clarithromycin and quinalizarin. Chinese J Spectrosc Lab 2005;2:356-9.
37. Hamdan II, Mishal AM. A spectroscopic method for quantitative determination of clarithromycin and roxithromycin. Saudi Pharm J 2000;8:191-7.
38. Ghoneim MM, El-Attar MA. Adsorptive stripping voltammetric determination of antibiotic drug clarithromycin in bulk form, pharmaceutical formulation and human urine. Chem Anal (Warsaw) 2008;53:689-702.
39. She-Ying D, Xiao-Feng H, Ting-Lin H, Xue-Man Y. Investigation on determination of clarithromycin by differential pulse voltammetry. Chin J Pharm Anal 2007;27:339-41.
40. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Complementary Guideline on Methodology, London; 2005.
41. Ashour S, Chehna MF, Bayram R. Spectrophotometric determination of alfuzosin HCl in pharmaceutical formulations with some sulphonephthalein dyes. Int J Biomed Sci 2006;2:273-8.
42. The Ministry of Health. Japanese Pharmacopoeia. 15th ed. Tokyo: The Ministry of Health, Labour and Welfare; 2006.
43. Amin AS, Gouda AAEF, El-Sheikh R, Zahran F. Spectrophotometric determination of gatifloxacin in pure form and in pharmaceutical formulation. Spectrochim Acta A Mol Biomol Spectrosc 2007;67:1306-12.