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Spectrophotometric Methods for Simultaneous Determination of Rivaroxaban and Clopidogrel in Their Binary Mixture

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Abstract

Three rapid, accurate and very simple derivative spectrophotometric methods for RIV and CLP assay in their binary mixture and tablet dosage forms were developed. Method (I) is first derivative spectrophotometric method, derivative amplitudes were measured at the zero crossing wavelength of 289 and 249.5 nm for estimation of RIV and CLP, respectively. The linearity is over the range 2.0 - 20.0 µg/ml for RIV and 5.0 - 60.0 µg/ml for CLP with LOD of 0.211 and 0.361 µg mL^{-1} and LOQ of 0.641 and 1.095 µg mL^{-1} for RIV and CLP, respectively. Method (II) is ratio derivative spectrophotometric method. The ratio spectra of each drug were derived by dividing its spectra on a constant concentration of the other drug as a divisor. Derivative amplitudes were measured at 256 nm for RIV and at 214.5 nm for CLP over the same linearity range as the first method with LOD of 0.137 and 0.485 µg mL^{-1} and LOQ of 0.417 and 1.471 µg mL^{-1} for RIV and CLP, respectively. Method (III) is absorbance ratio method, absorbance of both drugs were recorded at two wavelengths λ_1 (232) iso-absorptive point and λ_2 (249) λ_{max} of RIV. The final concentrations were obtained by applying the Q equations. The method was linear over the same concentration range as the first method with LOD of 0.272 and 0.485 µg mL^{-1} and LOQ of 0.826 and 1.471 µg mL^{-1} for RIV and CLP, respectively. The proposed methods were validated as per ICH guidelines. The proposed methods were successfully applied to both drugs analysis in their laboratory prepared co formulated tablet. Statistical comparison of the obtained results with those of the reference method show good agreement and confirm that there were no significant difference in the accuracy and precision between the proposed and reference one respectively.

Keywords: Rivaroxaban (RIV); Clopidogrel (CLP); Spectrophotometric methods; Binary mixture

Introduction

Rivaroxaban (Figure 1a); (S)-5-chloro-N-{[2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl] oxazolidin-5-yl]methyl} thiophene-2-carboxamide [1], is considered one of the available orally active anticoagulants, its mechanism of action was direct factor Xa inhibitor. (FDA) approved rivaroxaban to avoid occurrence of deep vein thrombosis which may cause pulmonary embolism in adults with hip and knee replacement surgery [2]. Clopidogrel (Figure 1b); ((+)-(S)-methyl2-(2-chlorophenyl)-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)yl)acetate [1], is an oral anticoagulant agent belongs to thienopyridine – class acts by inhibiting adenosine diphosphate-mediated platelet aggregation [2]. Both drugs are used to prevent blood clots in coronary artery disease, peripheral and cerebro vascular disease and to avoid myocardial infarction [2]. RIV is not official in any pharmacopoeia. Few spectrophotometric methods have been reported for determinations of RIV [3,4]. Two HPTLC methods were explored for the evaluation of RIV in tablet dosage form [5,6]. Many HPLC methods were reported in literature for RIV assay in pure form, pharmaceutical formulations and biological matrices [7-13]. For CLP, it is official in the United State Pharmacopeia (USP) [14] and British Pharmacopeia (BP) [15]. Literature survey reveals many reported spectrophotometric methods for CLP determination [16-19]. Many HPLC methods were published for its evaluation [20-23]. The present work provides three optimized and validated spectrophotometric methods for simultaneous determination of both drugs showing high simplicity. Validation for the proposed methods was done as per International Conference on Harmonization (ICH) guidelines.

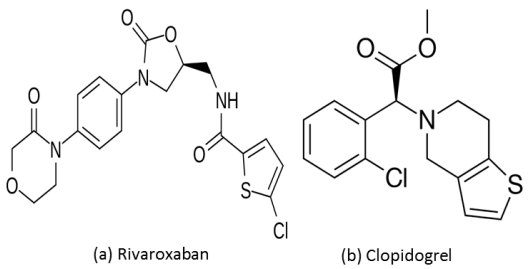

Figure 1: The structural formulae of the studied drugs.

Experimental Study

Equipments

The Spectrophotometric measurements were carried out using Shimadzu UV-Visible double-beam 1601 recording Spectrophotometer (Kyoto, Japan) (P/N 206-67001); with matched 1 cm path-length quartz cuvettes.

The first derivative spectra of both drugs were scanned in the wavelength range (200 - 320) nm with $\Delta\lambda$ = 8 nm and applying scaling factor = 10.

The ratio derivative spectra for RIV were recorded in the wavelength range (235-284) nm applying $\Delta\lambda$ = 2 nm and scaling factor = 1 to produce a smooth ratio spectra and $\Delta\lambda$ = 4 nm for the first derivative of ratio spectra appling scaling factor = 10. Meanwhile,

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the ratio derivative spectra for CLP were recorded in the wavelength range (200-280) nm with Δλ = 2 nm and scaling factor = 1 and Δλ = 4 nm for the first derivative of the produced ratio spectra with scaling factor = 10.

Ultrasonic bath (Model: SS 101 H 230, USA) used for sonication of all prepared solutions.

Materials and reagents

-Rivaroxaban and Clopidogrel were purchased from Memphis Co. for pharm. & Eva Pharma Company, Cairo, Egypt, respectively.

-Laboratory prepared tablet consists of 15 mg RIV, 75 mg CLP, 15 mg maize starch, 15 mg lactose, 7 mg magnesium stearate and 20 mg talc powder.

-Methanol was purchased from Sigma- Aldrich (Germany) as HPLC grade.

Standard solutions

Standard stock solutions of 10.0 mg/100 ml for each of Rivaroxaban and Clopidogrel were prepared separately in methanol. Using methanol for dilution of the stock solutions, working solutions were appropriately prepared. An additional stock solution was prepared using 50 mg/100 ml of Clopidogrel to be used in synthetic mixture preparation. The drugs kept stable for not less than 3 weeks without alteration when stored in the refrigerator.

Calibration graphs establishment

D^1 method: A series of 10 ml volumetric flasks was used. Transfer accurately measured volumes of the drug working standard solutions to reach final concentration in the range of 2.0 -20.0 µg/ml for RIV and 5.0-60.0 µg/ml for CLP. Dilute with methanol to the volume and mix well. RIV and CLP zero-order absorption spectra were recorded against blank methanol (Figure 2). The first derivatives of the zero-order spectra were recorded. The derivative amplitudes were measured at the zero crossing wavelength of 289 and 249.5 nm for determination of RIV and CLP, respectively (Figures 3 and 4). A plot of derivative absorbance amplitudes was then drawn against the final concentrations. Also, we derived the corresponding regression equations from the obtained data.

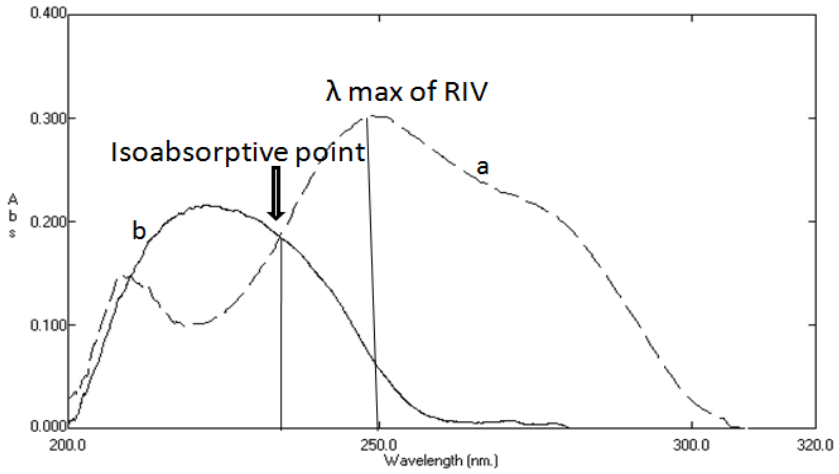

Figure 2: Zero order absorption spectra of RIV (a; 5.0 µg/ml) and CLP (b; 10.0 µg/ml) in methanol.

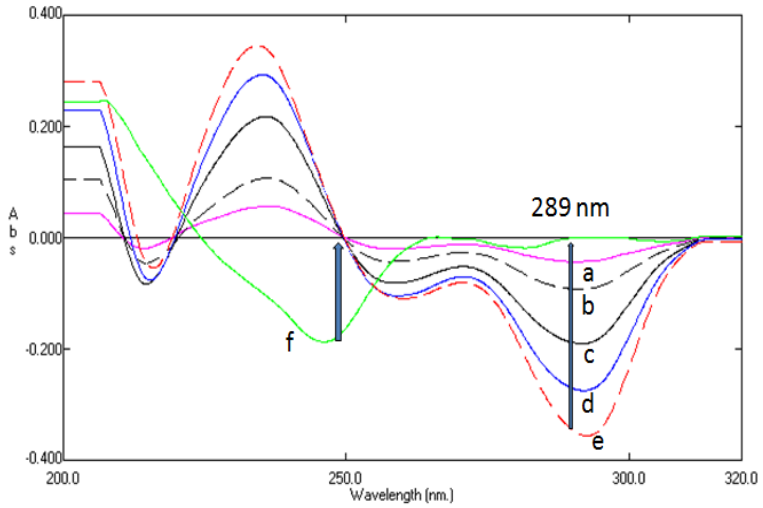

Figure 3: First order absorption spectra of methanolic solution of RIV (a: e) (2.0, 5.0, 10.0, 15.0, 20.0 µg/mL) and CLP (f) (20.0 µg/mL).

Ratio derivative spectrophotometry: In case of using ratio derivative spectrophotometry, the first derivative of the ratio spectra (all RIV spectra were divided by CLP spectrum (20.0 µg/mL) (Figures 5 and 6) and all CLP spectra were divided by RIV spectrum of 5.0 µg/mL) were derived (Figures 7 and 8). After measuring the amplitudes at 256 nm for RIV and at 214.5 nm for CLP, the derivative amplitudes were plotted against the final concentrations to obtain the calibration curves.

Absorbance ratio method: Determination of drugs by absorbance ratio method was also derived, absorbance of both drugs were recorded at two wavelengths λ_1 (232) iso-absorptive point and λ_2 (249) λ_{max} of RIV (Figure 2). The final concentrations were obtained by applying the Q equations.

Application of the proposed methods (I, II, III) to RIV/CLP binary mixtures assay

Accurate aliquots of RIV and CLP standard solutions in their pharmaceutical ratio (1:5) were transferred into a series of 10 mL volumetric flasks. The solutions were diluted with methanol to the volume and then mixed well. The steps stated under "Calibration Graphs Establishment" (a, b, c) were then followed. Graphs of derivative amplitudes against the final concentrations were plotted to obtain the linearity range.

proposed method application for determination of riv and clp in their laboratory prepared co-formulated tablets

Ten laboratory prepared tablets were mixed well, then amount equivalent to 15 mg RIV and 75 mg CLP were accurately weighed and transferred into 100 mL volumetric flask and about 80 mL of methanol were added. Sonication of the flask contents for 30 min was applied, and then the flask was completed to the volume with the same solvent and filtered. Aliquot volumes of the filtrate of suitable concentrations in the working concentration ranges for both studied drugs were assayed as illustrated under "Calibration Graphs Establishment". Tablet contents were calculated using calibration graph or corresponding regression equations.

Results and Discussion

RIV UV spectrum showed maxima at 210 and 248 nm and for

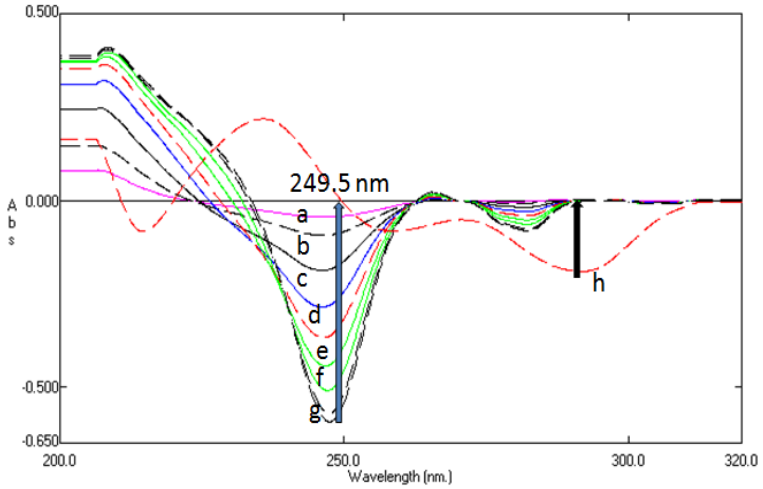

Figure 4: First order absorption spectra of methanolic solution of CLP (a: g) (5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0 µg/mL) and RIV (h) (10.0 µg/mL).

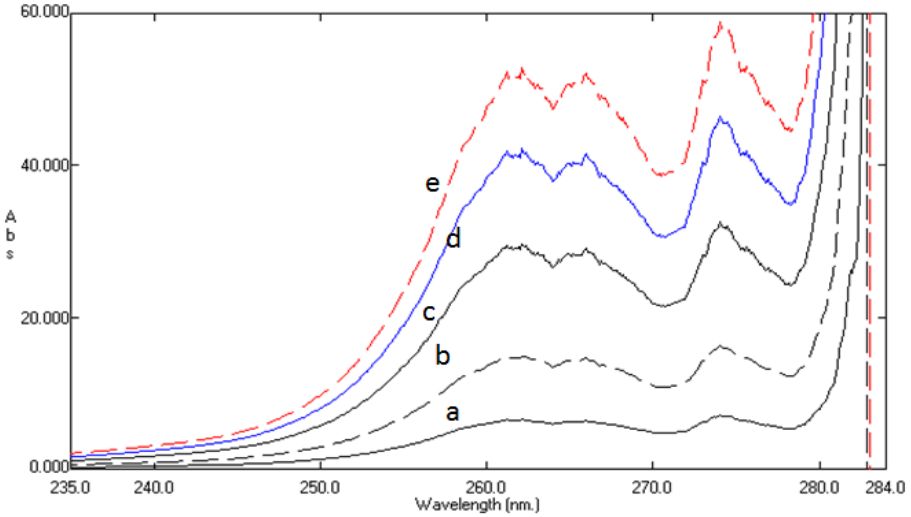

Figure 5: Ratio spectra of RIV (a: e) divided by CLP (20.0 µg/mL) as a divisor in methanol.

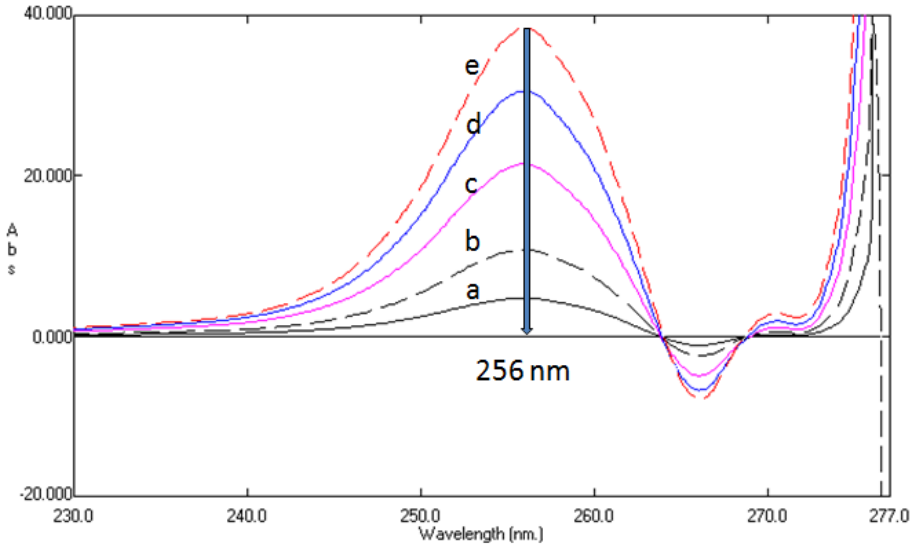

Figure 6: Ratio derivative spectra of RIV (a: e) divided by CLP (20.0 µg/mL) as a divisor in methanol.

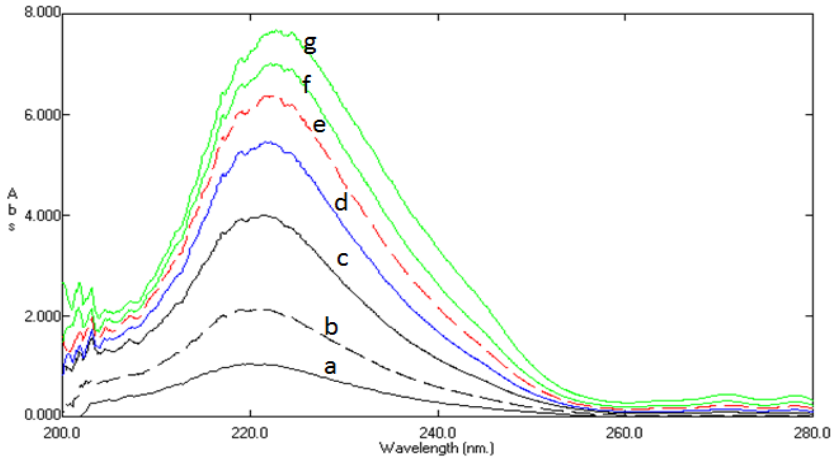

Figure 7: Ratio spectra of CLP (a: g) divided by RIV (5.0 µg/mL) as a divisor in methanol.

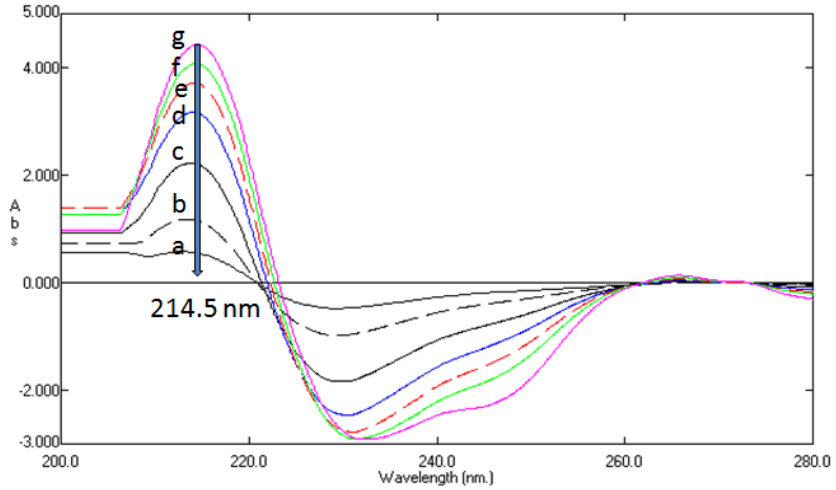

Figure 8: Ratio derivative spectra of CLP (a: g) divided by RIV (5.0 µg/mL) as a divisor in methanol.

CLP 222 nm (Figure 2). Due to this overlap, RIV can be evaluated in presence of CLP in the zero order spectra but the readings were of low sensitivity at 275 nm and CLP cannot be estimated in presence of RIV. Thus, we developed three spectrophotometric methods (derivative and ratio derivative and ratio absorbance) for the quantification of both drugs in the presence of each other.

First derivative spectrophotometric method

The use of derivative spectrophotometric technique make enhancement for the UV absorption spectrum features; the first derivative spectra of both RIV and CLP (Figures 3 and 4) permitted more specific and highly selective determination of each drug at the zero-crossing point of the other. The first derivative amplitudes at 289 nm (zero-crossing of CLP) and at 249.5 nm (zero-crossing of RIV) were selected for assay of RIV and CLP in their binary mixtures, respectively.

Ratio derivative spectrophotometry

Figure 5 illustrates the ratio spectra of RIV standards in different concentrations (RIV spectra divided by 20.0 µg/mL of CLP spectrum) while Figure 6 shows their corresponding first derivative spectra. In this figure, the amplitude at 256 nm ($^1DD_{256}$) corresponds to RIV present in the solution, so it can be used for its quantitative assay.

Likewise, Figure 7 and 8 shows the ratio spectra of different concentrations of CLP standards (spectra divided by the spectrum of 5.0 µg/mL RIV solution) as well as their corresponding first derivative spectra, on the basis of which, CLP can be estimated by measuring the amplitude at 214.5 nm ($^1DD_{214.5}$).

The effect of Δλ for obtaining the first derivative of the ratio spectra was tested to obtain the best wavelength interval; Δλ = 8 nm was considered as suitable for both drugs. CLP (20.0 µg /ml) and RIV (5 µg /ml) were selected as the most suitable divisor concentration in relation to signal-to-noise ratio and sensitivity for evaluation of RIV and CLP, respectively.

Absorbance ratio method

Figure 2 shows the iso-absorptive point at which both drugs have the same absorbance value and λ max for RIV. Absorbance of each drug was measured twice; at the iso-absorptive point and at λ max for RIV, then calibration for each drug alone was performed and specific absorptivities of both drugs were determined.

Relative concentration of the two drugs in the mixture was calculated by applying the following equations [24].

$$C_x = [(Q_M - Q_y)/ (Q_x - Q_y)] \times A_1/a_{x1}$$

$$C_y = [(Q_M - Q_x)/ (Q_y - Q_x)] \times A_1/a_{y1}$$

Where $Q_M = A_2$ (absorbance of the sample at 248.5 nm)/ A_1 (absorbance of the sample at 233.7 nm), $Q_x = a_{x2}$ (absorptivity of RIV at 248.5 nm)/ a_{x1} (absorptivity of RIV at 233.7 nm), $Q_y = a_{y2}$ (absorptivity of CLP at 248.5 nm) / a_{y1} (absorptivity of CLP at 233.7 nm).

A_1 and A_2 are the absorbance of the mixture at 233.7 nm and 248.5 nm, respectively.

a_{x1} and a_{y1} are the absorptivities of RIV and CLP at 233.7 nm, a_{x2} and a_{y2} are the absorptivities of RIV and CLP at 248.5 nm.

Proposed Methods Validation

Linearity and range

A plot of the derivative amplitude against the drug concentration in µg/ml showed a linear relationship over the range 2.0–20.0 µg/mL for RIV and 5.0-60.0 µg/mL for CLP. The concentration ranges over which linearity was cited in Tables 1 and 2.

The following equations were obtained from data regression analysis:

DA = 0.010+0.017C (r = 0.9999) for RIV at 289 nm.

DA = 0.005+0.008C (r = 0.9999) for CLP at 249.5 nm.

The ratio first derivative method resulted in the following equations:

DA = 0.010+0.017C (r = 0.9999) for RIV at 256 nm.

DA = 0.005+0.008C (r = 0.9999) for CLP at 214.5 nm.

Illustration: DA refers to the derivative amplitude, C refers to drug concentration in µg/mL and correlation coefficient is r.

High (r) values and small values of intercepts prove that calibration curves were of high linearity.

Q equations for absorbance ratio method were represented as follow:

$$C_x = [(Q_M - 0.4228)/ (1.5401 - 0.4228)] \times A_1/0.0337$$

$$C_y = [(Q_M - 1.5401)/ (0.4228 - 1.5401)] \times A_1/0.0149$$

The linearity of absorbance ratio method was performed for each drug twice (at iso-absorptive point and at λ_{max} of RIV).

From data of statistical analysis [24,25] it was found that the

Parameter	Results	
	RIV	CLP
Linearity range (µg/mL)	2.0 - 20.0	5.0 - 60.0
(a)	0.01	0.005
(b)	0.017	0.008
(r)	0.9999	0.9999
(Sy/x)	1.3×10^{-3}	1.2×10^{-3}
(Sa)	1.1×10^{-3}	9.0×10^{-4}
(Sb)	1.0×10^{-4}	0
% RSD	0.69	0.73
% Error	0.31	0.27
LOD (µg/mL)	0.211	0.361
LOQ (µg/mL)	0.641	1.095

Table 1: The determination of the RIV and CLP analytical performance using the proposed first derivative spectrophotometric method 3.

Parameter	Results	
	RIV	CLP
Linearity range (µg/mL)	2.0 - 20.0	5.0 - 60.0
(a)	1.02	0.59
(b)	1.94	0.082
(r)	0.9999	0.9999
(Sy/x)	9.6×10^{-2}	1.68×10^{-2}
(Sa)	0.08	1.21×10^{-2}
(Sb)	6.6×10^{-3}	3.0×10^{-4}
% RSD	0.41	0.96
% Error	0.18	0.36
LOD (µg/mL)	0.137	0.485
LOQ (µg/mL)	0.417	1.471

Table 2: The determination of the RIV and CLP analytical performance using the proposed ratio first derivative spectrophotometric method.

correlation coefficients (r) were of high values indicating high linearity of the proposed methods and the results obeys Beer's law.

Low scattering of the points around the calibration curves was predicted from the small values of the standard deviation of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b). Owing to small values of percentage relative standard deviation (RSD%) and percentage relative errors (%Er), the proposed method illustrates high accuracy and high precision.

Limit of detection (LOD) and limit of quantitation (LOQ)

Quantitation limit (LOQ) and detection limit (LOD) were determined according to ICH recommendations [26] using the following equation.

$LOQ = 10\ S_a/b$ $LOD = 3.3\ S_a/b$

Where: S_a is standard deviation of the intercept of the calibration curve and, b is the slope of the calibration curve.

Table 1 shows that: 0.64 and 1.09 µg/mL were LOQ values while 0.21 and 0.36 µg/mL were LOD values for RIV and CLP, respectively for first derivative spectrophotometric method. Table 2 illustrates that: 0.42 and 1.47 µg/mL were LOQ values while 0.14 and 0.48 µg/mL were LOD values for RIV and CLP, respectively for ratio first derivative spectrophotometric method. Table 3 illustrates that: 0.826 and 1.471µg/mL were LOQ values while 0.272 and 0.485 µg/mL were LOD values for RIV and CLP, respectively for absorbance ratio spectrophotometric method.

Method accuracy

The analysis of the obtained results of the proposed methods for the RIV and CLP were compared with those produced using the reference method [13] for proving the accuracy of our proposed method. The obtained results were analyzed statistically using Student's t-test and variance ratio F-test [25] which illustrated no significant difference between both methods performance regarding accuracy and precision, respectively. Student's t-test and variance ratio F-test are presented in Table 4.

-The precision for proposed methods was determined by (intra-day) and (inter-day) assay for both drugs.

Parameter	Results			
	RIV		CLP	
Wavelength (nm)	233.7	248.5	233.7	248.5
Linearity range (µg/mL)	2.0 - 20.0		5.0 - 60.0	
Regression equation	Y = 0.0138 + 0.0337X	Y = 0.0466 + 0.0519X	Y = 0.0435 + 0.0149X	Y = 0.0061 + 0.0063X
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
S.D. of residuals (Sy/x)	3.3×10^{-3}		3.0×10^{-3}	
S.D. of intercept (Sa)	2.8×10^{-3}		2.2×10^{-3}	
S.D. of slope (Sb)	2.0×10^{-4}		1.0×10^{-4}	
Percentage relative standard deviation, % RSD	1.02	0.74	0.79	0.67
Percentage relative error, % Error	0.45	0.33	0.29	0.25
Mean	99.78	99.85	99.95	99.39
Detection limit, LOD (µg/mL)	0.272		0.485	
Quantitation limit, LOQ (µg/mL)	0.826		1.471	

Table 3: The determination of the RIV and CLP analytical performance by the proposed absorbance ratio spectrophotometric method.

Intra-day precision: The three proposed methods were subjected to intra-day precision through replicate analysis of three concentrations of both tested drugs on three different times within the same day. The obtained results showed small values of % Error and % RSD indicate high accuracy and precision of the proposed methods, respectively. The outcomes are cited in Tables 5-7.

Inter-day precision: Inter-day precision was carried out through replicate analysis of three concentrations of the studied drugs on three successive days. The results are stated in Tables 5-7.

Proposed method	Analyte	Results			Reference method [13]
		Amount taken (µg/mL)	Amount found (µg/mL)	% Found	% Found
		2.0	1.976	98.84	98.77
		5.0	5.000	100.00	100.44
First derivative spectrophotometry	RIV	10.0	10.058	100.58	99.90
		15.0	14.883	99.22	
		20.0	20.000	100.00	
	x ± SD			99.73 ± 0.69	99.70 ± 0.85
	t-test			0.05 (2.45)	
	F-test			1.51 (6.94)	
	CLP	5.0	4.9259	98.52	98.48
		10.0	9.9877	99.88	100.55
		20.0	19.9877	99.94	99.88
		30.0	30.2346	100.78	
		40.0	40.2346	100.59	
		50.0	49.987	99.98	
		60.0	59.987	99.98	
	x ± SD			99.95 ± 0.73	99.64 ± 1.06
	t-test			0.56 (2.31)	
	F-test			2.12 (5.14)	
Ratio first derivative spectrophotometry		Amount taken (µg/mL)	Amount found (µg/mL)	% Found	% Found
		2.0	1.989	99.47	98.77
		5.0	4.978	99.57	100.44
	RIV	10.0	10.020	100.21	99.9
		15.0	15.062	100.42	
		20.0	19.950	99.75	
	x ± SD			99.88 ± 0.41	99.70 ± 0.85
	t-test			0.41 (2.45)	
	F-test			4.26 (6.94)	
	CLP	5.0	4.942	98.86	98.48
		10.0	9.814	98.15	100.55
		20.0	20.070	100.35	99.88
		30.0	30.180	100.60	
		40.0	40.289	100.72	
		50.0	50.034	100.07	
		60.0	59.778	99.63	
	x ± SD			99.77 ± 0.96	99.64 ± 1.06
	t-test			0.19 (2.31)	
	F-test			1.22 (5.14)	

		Amount taken (µg/mL)	Amount found (µg/mL)	% Found	% Found
Absorbance ratio spectrophotometry		2.0	1.964	98.22	98.77
		5.0	4.991	99.82	100.44
	RIV	10.0	10.035	100.36	99.90
		15.0	15.139	100.93	
		20.0	19.916	99.58	
	x ± SD			99.78 ± 1.02	99.70 ± 0.85
	t-test			0.11 (2.45)	
	F-test			1.42 (19.24)	
		5.0	4.9329	98.66	98.48
		10.0	9.9664	99.66	100.55
	CLP	20.0	19.9664	99.83	99.88
		30.0	30.3691	101.23	
		40.0	40.1678	100.42	
		50.0	49.832	99.66	
		60.0	60.100	100.17	
	x ± SD			99.95 ± 0.79	99.64 ± 1.06
	t-test			0.52 (2.31)	
	F-test			1.78 (5.14)	

Table 4: Assay results for RIV and CLP determination in their pure form by the three proposed spectrophotometric and reference methods.

Concentration added (µg/ml)	% Found ± SD	% RSD	% Error
RIV			
Intra-day			
2	99.65 ± 0.95	0.95	0.55
5	100.34 ± 0.72	0.72	0.41
10	99.35 ± 0.91	0.91	0.53
Inter-day			
2	99.22 ± 1.00	1	0.58
5	100.08 ± 0.68	0.67	0.39
10	100.23 ± 0.88	0.88	0.51
CLP			
Intra-day			
10	100.13 ± 0.86	0.86	0.5
25	100.18 ± 0.94	0.94	0.54
50	99.73 ± 0.61	0.61	0.35
Inter-day			
10	99.33 ± 1.07	1.08	0.62
25	99.71 ± 0.83	0.83	0.48
50	100.44 ± 0.68	0.68	0.39

Table 5: Precision results for the determination of RIV and CLP by the proposed first derivative spectrophotometric method.

The small values of % Error and % RSD indicate high accuracy and precision of the proposed method, respectively.

Specificity

Common tablet excipients may interfere with the assay of the drugs which affect the specificity of the method. The excipients did not interfere with the results of our proposed methods indicating high specificity of the method. The specificity of the proposed method was investigated by viewing any interference of common tablets excipients such as talc powder, lactose, maize starch, magnesium stearate, calcium hydrogen phosphate and microcrystalline cellulose.

Concentration added (µg/ml)	% Found ± SD	% RSD	% Error
RIV			
Intra-day			
2	98.99 ± 0.72	0.72	0.61
5	100.00 ± 0.65	0.65	0.54
10	99.14 ± 0.93	0.93	0.59
Inter-day			
2	100.08 ± 0.53	0.53	0.51
5	99.26 ± 0.68	0.68	0.49
10	100.20 ± 0.58	0.58	0.52
CLP			
Intra-day			
10	100.24 ± 0.76	0.76	0.5
25	101.00 ± 0.84	0.84	0.56
50	99.80 ± 0.67	0.67	0.45
Inter-day			
10	99.18 ± 0.84	0.84	0.61
25	100.25 ± 0.73	0.73	0.47
50	100.04 ± 0.66	0.66	0.38

Table 6: Precision data for RIV and CLP assay by the proposed ratio first derivative spectrophotometric method.

Concentration added (µg/ml)	% Found ± SD	% RSD	% Error
RIV			
Intra-day			
2	99.60 ± 0.49	0.49	0.64
5	100.18 ± 0.71	0.71	0.44
10	99.15 ± 0.88	0.88	0.56
Inter-day			
2	100.02 ± 0.62	0.62	0.53
5	99.55 ± 0.74	0.74	0.6
10	100.43 ± 0.56	0.56	0.55
CLP			
Intra-day			
10	100.00 ± 0.77	0.77	0.52
25	99.75 ± 0.85	0.85	0.57
50	99.86 ± 0.66	0.66	0.47
Inter-day			
10	99.44 ± 0.90	0.9	0.6
25	100.05 ± 0.74	0.74	0.39
50	100.54 ± 0.55	0.55	0.51

Table 7: Precision results for RIV and CLP determination by the proposed absorbance ratio spectrophotometric method.

RIV/CLP ratio	Proposed method					
	Amount taken (µg/ml)		Amount found (µg/ml)		% Found	
	RIV	CLP	RIV	CLP	RIV	CLP
1:5	2.0	10.0	1.9745	10.00	98.73	100.00
	5.0	25.0	5.0357	24.82	100.71	99.29
	10.0	50.0	9.9847	49.82	99.85	99.64
Mean					99.76	99.64
± SD					± 0.99	± 0.36
% RSD					0.99	0.36
% Error					0.57	0.21

Table 8: Results for RIV and CLP determination in synthetic mixtures in ratios of 1:5 (w/w) by the proposed first derivative method.

RIV/CLP ratio	Proposed method					
	Amount taken (µg/ml)		Amount found (µg/ml)		% Found	
	RIV	CLP	RIV	CLP	RIV	CLP
1:5	2.0	10.0	1.9841	9.8799	99.21	98.80
	5.0	25.0	5.0254	25.192	100.51	100.77
	10.0	50.0	9.9904	49.927	99.9	99.85
					99.87	99.81
± SD					± 0.65	± 0.99
% RSD					0.65	0.99
% Error					0.37	0.57

Table 9: Results for RIV and CLP determination in synthetic mixtures in ratios of 1:5 (w/w) by the proposed ratio first derivative method.

RIV/CLP ratio	Proposed method					
	Amount taken (µg/ml)		Amount found (µg/ml)		% Found	
	RIV	CLP	RIV	CLP	RIV	CLP
1:5	2.0	10.0	1.967	10.100	98.35	101.00
	5.0	25.0	5.010	24.89	100.2	99.56
	10.0	50.0	10.030	49.840	100.30	99.68
					99.62	100.08
± SD					± 1.09	± 0.79
% RSD					1.09	0.79
% Error					0.63	0.46

Table 10: Results for RIV and CLP determination in synthetic mixtures in ratios of 1:5 (w/w) by the proposed absorbance ratio method.

Robustness

The robustness of the method was assessed by evaluating the influence of small variations in the experimental variables on the analytical performance of the method. The small variations in any of the variables did not significantly affect the results; percentage recovery was 99.78 ± 1.02 and 99.95 ± 0.79 for RIV and CLP respectively. This provided an indication of the reliability of the proposed methods during routine work.

Ruggedness

For expressing the stability of the method against extraneous influencing factors such as analyst, laboratory, instrument. The proposed method provided stable results in different laboratories which indicate reliability of the proposed method.

Application of the Proposed Methods to the Determination of RIV/CLP in Laboratory-Prepared Mixtures

Simultaneous evaluation of RIV and CLP in laboratory prepared binary mixtures in their pharmaceutical ratio; 1:5 was performed as an application of the proposed methods. In the laboratory prepared mixtures, the concentrations taken of both drugs were presented in the linearity range. The values of %RSD and %Er were calculated and the results indicate high accuracy of the proposed method as shown in Tables 8-10.

Laboratory prepared co-formulated tablets (RIV 15mg +CLP 75mg)	First derivative spectrophotometric method						Reference method [13]	
	Amount taken (µg/ml)		Amount found (µg/ml)		% Found		% Found	
	RIV	CLP	RIV	CLP	RIV	CLP	RIV	CLP
	2.0	10.0	2.000	9.9394	100.00	99.39	99.37	100.47
	5.0	25.0	5.026	25.0909	100.53	100.36	100.47	99.65
	10.0	50.0	10.026	49.9394	100.26	99.88	99.92	100.06
Mean					100.26	99.88	99.92	100.06
± SD					± 0.27	± 0.49	± 0.55	± 0.41
% RSD					0.27	0.49	0.55	0.41
% Error					0.15	0.28	0.32	0.24
Student t test					0.97	0.5		
F					4.31	1.39		

Table 11: Assay results for RIV and CLP determination in their laboratory prepared tablet by the proposed first derivative spectrophotometric and reference method.

Laboratory prepared co-formulated tablets (RIV 15 mg +CLP 75 mg)	Ratio first derivative spectrophotometric method						Reference	
	Amount taken (µg/ml)		Amount found (µg/ml)		% Found		% Found	
	RIV	CLP	RIV	CLP	RIV	CLP	RIV	CLP
	2.0	10.0	1.983	9.9211	99.19	99.21	99.37	100.47
	5.0	25.0	5.026	25.1384	100.52	100.55	100.47	99.65
	10.0	50.0	9.99	49.9668	99.9	99.93	99.92	100.06
Mean					99.87	99.9	99.92	100.06
± SD					± 0.67	± 0.67	± 0.55	± 0.41
% RSD					0.67	0.67	0.55	0.41
% Error					0.38	0.38	0.32	0.24
Student t test					0.1	0.36		
F					1.46	2.67		

Table 12: Results of RIV and CLP assay in their laboratory prepared tablet by the proposed ratio first derivative spectrophotometric and reference method.

Laboratory prepared co-formulated tablets (RIV 15 mg +CLP 75 mg)	Ratio first derivative spectrophotometric method						Reference	
	Amount taken (µg/ml)		Amount found (µg/ml)		% Found		% Found	
	RIV	CLP	RIV	CLP	RIV	CLP	RIV	CLP
	2.0	10.0	2.003	9.892	100.15	98.92	99.37	100.47
	5.0	25.0	5.061	25.040	101.22	100.16	100.47	99.65
	10.0	50.0	9.950	50.00	99.50	100.00	99.92	100.06
Mean					100.29	99.69	99.92	100.06
± SD					± 0.87	± 0.67	± 0.55	± 0.41
% RSD					0.87	0.67	0.55	0.41
% Error					0.50	0.39	0.32	0.24
Student t test					0.62	0.80		
F					2.49	2.71		

Table 13: Assay results for RIV and CLP determination in their laboratory prepared tablet by the proposed absorbance ratio spectrophotometric and reference method.

Application of the Proposed Methods to the Determination of the Studied Drugs in Laboratory Prepared Co-formulated Tablets

Our proposed methods were applied for the estimation of both tested drugs in their prepared tablets. Tables 11-13 showed that the results obtained are in good agreement with the results of the comparison one [13]. The obtained data were statistically analyzed using Student's t-test and variance ratio F-test [24] showing no significant difference between the performance of the two methods, so our method is of high accuracy and precision.

Conclusion

RIV and CLP binary mixtures were analyzed using an accurate, sensitive, and rapid three spectrophotometric methods. Also, the studied drugs could be determined in prepared tablets using the proposed spectrophotometric procedures. These proposed methods can be applied in quality control laboratories, science it showed good validation criteria.

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