SPECTROPHOTOMETRIC DETERMINATION OF RIFAMPICIN IN BULK DRUG AND PHARMACEUTICAL FORMULATIONS BASED ON REDOX AND COMPLEXATION REACTIONS [**]

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Two spectrophotometric methods were developed and validated for the determination of rifampicin (RIF) in bulk form, formulations, and spiked human urine. The first method is based on the reduction of the Folin–Ciocalteu (FC) reagent by RIF to form a blue colored chromogen with λ_{max} at 760 nm (the FCR method). In the second method, iron(III) is reduced by RIF in a neutral medium, and the resulting iron(II) is complexed with ferricyanide to form a Prussian blue peaking at 750 nm (the FFC method). Under optimum conditions, Beer's law enabled the determination of the drug in the concentration ranges 1–35 and 2.5–50 μg/mL with apparent molar absorptivities of 2.72×10^4 and 1.63×10^4 L/(mol · cm) for the FCR and FFC methods, respectively. The Sandell sensitivity, limits of detection (LOD), and quantification (LOQ) values were also reported for both methods. The precision of the methods, with % RSD of < 2%, was satisfactory, and the accuracy was higher than 2% (RE). The proposed methods were successfully applied to the determination of drug in capsules without interference from common additives and spiked human urine without interference from endogenous substances. A statistical analysis indicated that there was no significant difference between the results obtained by the developed methods and the official method.

Keywords: *rifampicin, assay, spectrophotometry, FC reagent, ferricyanide.*

СПЕКТРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ РИФАМПИЦИНА В ОБЪЕМНОЙ ЛЕКАРСТВЕННОЙ ФОРМЕ НА ОСНОВЕ РЕДОКСА И ОБРАЗОВАНИЯ КОМПЛЕКСОВ

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Разработаны и протестированы два спектрофотометрических метода определения рифампицина (RIF) в объеме, составе и отдельной порции человеческой урины. Первый метод основан на восстановлении реагента Folin-Ciocalteu (FC) RIF с образованием синего хромогена с λ_{max} = 760 нм (метод FCR). Во втором методе железо(III) восстанавливают с помощью RIF в нейтральной среде; полученное железо(II) образует комплекс с феррицианидом с максимумом λ = 750 нм (метод FFC). В оптимальных условиях методы FCR и FFC позволяют определить концентрацию препарата в диапазонах 1–35 и 2.5–50 мкг/мл с молярной поглощательной способностью 2.72×10^4 и 1.63×10^4 л/(моль ·см). Оценены чувствительность Санделла, пределы детектирования (LOD) и измерения (LOQ). Точность методов (погрешность <2%) удовлетворительная. Предложенные методы успешно использованы для определения лекарства в капсулах и человеческой моче. Статистический анализ показывает отсутствие существенных различий между результатами, полученными разработанными методами и официально используемым методом.

Ключевые слова: *рифампицин, анализ, спектрофотометрия, реагент FC, феррицианид.*

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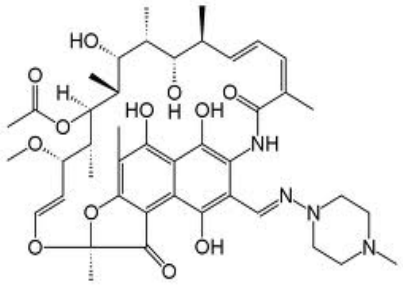
Introduction. Rifampicin (RIF) (Alignment in middle), chemically known as 3-[(4-methyl-1-piperazinyl)imino] methyl (rifamycin SV), is a semisynthetic antibiotic chemically derived from rifamycin SV [1, 2].



RIF

Rifamycin SV is a substance obtained by chemical transformation of rifamycin B, which is produced during the growth of certain strains of *stremptomyces mediterranei*, or isolated directly from a culture medium of certain *S mediterranei* mutants [2]. Rifamycin B and rifamycin SV, which are the parent antibiotics for the synthesis of rifampicin, as well as rifamycin S and rifamycin O, which are the impurities of rifamycin SV [1], differ in their chemical structure from rifampicin: rifamycin antibiotics do not contain the side chain of 3-[(4-methyl-1-piperazinyl)imino]methyl group. Being potentially hepatotoxic, RIF is an established first line antituberculosis agent, and its use in other serious infections (such as HIV) is expanding [3]. It is metabolized in the liver mainly by deacetylation. Hence, when RIF is used for patients with liver diseases, RIF concentration monitoring is absolutely necessary [4, 5].

Different techniques have been developed for the RIF concentration measurement in biological fluids: fluorimetry [6], liquid chromatography HPLC [7, 8], nuclear magnetic resonance spectroscopy [9], cyclic and square wave voltammetry [10], and visible spectrophotometry [11, 12]. The drug officially undergoes a microbiological assay in India [13]. Several methods are available for the determination of RIF combined with other anti-tuberculosis drugs, namely multivariate spectrophotometry [14–16], direct UV-spectrophotometry [17], first-derivative UV-spectrophotometry [18–20], double-divisor ratio spectrometry [21], graphical absorbance ratio and absorbance additive spectrophotometry [18], HPLC [7, 22, 23], HPTLC [24, 25], cyclic and linear sweep voltammetry [26], cyclic and square wave voltammetry [10], differential pulse polarography [27, 28], and continuous flow chemiluminescence spectrometry [29, 30].

Several methods are also available for the determination of pure RIF in dosage forms. They are based on the use of horseradish peroxidase (HRP)-based amperometry [31], differential pulse polarography [32], differential pulse adsorptive stripping voltammetry [33], nuclear magnetic resonance spectroscopy (NMR) [34], and chemiluminescence spectrometry [35].

Visible spectrophotometry is an attractive technique due to its simplicity, adaptability, and reasonable sensitivity with significant economic advantages. Therefore, several spectrophotometric methods based on diverse reaction chemistries are known for RIF. Sadegi and Karimi [11] developed methods based on the charge-transfer (CT) complex formation reaction of RIF with three π-electron acceptors in an acetonitrile medium. RIF was determined by Sastry et al. [12] in pharmaceuticals by treating a solution of the drug with $ZrOCl_2$, $La(NO_3)_2$, $NiCl_2$, or $CeCl_3$ and pyridine followed by measurement of the absorbance of the resulting ternary complex. Two methods based on chelate formation with Cu^{2+} and CT complexation with halogenated quinones have been described by Shereen et al. [36]. Based on a similar reaction but using chloranil as a π-acceptor, Sastry et al. [37] determined RIF based on its reaction in a pH 7 buffer solution and measurement of the absorbance at 500 nm. By reacting RIF with ammonium metavanadate in an acidic medium, a new method was developed by Shukla et al. [38]. Uranyl acetate or thorium nitrate formed colored complex with RIF in the presence of PrOH and was measured at 490 or 545 nm, as described by Sastry et al. [39]. In an indirect method developed by Barsoum et al. [17], the drug reacted with a measured excess of NBS, the unreacted oxidant was treated with KI and starch, and the resulting starch-I_2 complex was measured at 572 nm. Based on colored reactions with 2,6-dichloroquinone chlorimide or $K_2Cr_2O_7$, RIF was determined by measuring the absorbance either at 545 or 540 nm [40]. In a method reported by Diwakar et al. [41], the drug reacted with *p*-N,N-dimethylphenylenediamine and chloramine-T, the resulting chromogen was extracted into butyl alcohol, and the absorbance was measured at 520 nm. RIF was found to form a colored chelate with iron(III) extracted into chloroform and measured at 540 nm [11]. Reddy and Sastry [42] determined the drug by ion-pair extraction with two dyes alizarin violet 3B and alizarin brilliant violet R followed by the absorbance measurement at 560 nm. Based on colored complex formation with Cu(II) acetate or

Co(II) chloride, Gandhi et al. [43] reported two methods for the drug. Recently [44], chloranilic acid has been suggested as a π-acceptor for the spectrophotometric assay of the drug.

It is expected that the developed methods be simple, sensitive, and cost-effective. However, the reported visible spectrophotometric methods are deficient in one way or another and are less sensitive in a narrow linear dynamic range; they require extraction, strict pH control, longer contact time, and/or the use of an organic solvent as the reaction medium.

The main goal of this work was, therefore, to develop and validate simple, sensitive, accurate, and precise spectrophotometric methods able to overcome the drawbacks of the published methods. The methods are based on the reducing property of RIF and the use of Folin–Ciocalteu (FC) and iron(III)-ferricyanide as chromogenic reagents. The methods are demonstrated to be advantageous and competitive for RIF in dosage forms and human urine.

Experimental. Absorbance measurements were conducted using a Systronics model 166 digital spectrophotometer (Ahmedabad, India) equipped with 1 cm matched glass cuvettes.

Being of analytical reagent grade or chemically pure grade, all our chemicals and reagents were used without further purification, and double distilled water was used throughout the investigation.

A 1:1 aqueous solution was prepared by mixing accurately measured 50 mL of FC (Merck, Mumbai, India) with 50 mL of water.

Standard drug solution. RIF (99.9% purity) was provided by Lupin Limited, Tarapur, Maharashtra, India, and used as received. Capsules in two strengths (R-Cin 300 and R-Cin 450) (Lupin Limited, Chikaltana, Aurangabad, India) were purchased from local markets.

A stock solution containing 100 μg/mL RIF was prepared by dissolving 10 mg of the drug in water and diluting to 100 mL in a standard flask.

Preparation of calibration graph. FCR method. Different aliquots (0.1–3.5 mL) of 100 μg/mL RIF solution were transferred into a number of 10 mL calibrated flasks using a micro-burette, and the total volume was adjusted to 3.5 mL with water. To each flask, 3.0 mL of 1:1 FC reagent and the same amount of 20% Na_2CO_3 were successively added, and the volume was brought to 10 mL with water. The flasks were stoppered, the content mixed well, and after 5 min the absorbance of the blue colored chromogen was measured against the reagent blank at 760 nm.

FFC method. Into a number of 10 mL calibration flasks, aliquots (0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mL) of 100 μg/mL RIF standard solution equivalent to 2.5-50 μg/mL RIF were accurately transferred, and to each flask 0.5 mL of 0.01 M $FeCl_3$ was added followed by 1.5 mL of 0.05% potassium ferricyanide along with 1.0 mL of 0.02 M orthophosphoric acid, and the content was then diluted with water. After 5 min, the absorbance of the blue colored complex was measured at 750 nm against the reagent blank, similarly prepared.

A standard graph was prepared by plotting the absorbance *versus* the drug concentration, and the unknown concentration was computed from the respective regression equation derived using the absorbance-concentration data in both methods.

Procedure for capsules. The contents of twenty capsules were pooled and pulverized. The amount of capsule powder equivalent to 10 mg RIF was quantitatively transferred into 100 mL volumetric flasks. The content was shaken well with about 60 mL of water for 20 min and, the content was diluted to the mark with water. It was filtered using Whatman No 42 filter paper. First, a 10 mL portion of the filtrate was discarded, and then a 2.5 mL portion of the subsequent portion was subjected to analysis.

Procedure for placebo blank and synthetic mixture. A placebo blank of the composition [17]: urea (10 mg), sodium oxalate (15 mg), camphor (10 mg), glucose (10 mg), lactose (20 mg), sucrose (15 mg) and ascorbic acid (10 mg) was prepared by homogeneous mixing. Ten milligrams was treated with water, and its extract was prepared as described in the procedure for capsules. A 2.5 mL aliquot was subjected to analysis as usual.

Ten milligrams of the placebo and the same amount of pure RIF were mixed thoroughly and transferred quantitatively to a 100 mL volumetric flask; the extract was prepared as described in the procedure for capsules. A 2.5 mL aliquot was taken for assay following the general procedure.

Procedure for spiked human urine. The method of Salem et al. [9] was used to prepare a spiked urine sample. Ten milligrams of pure RIF and 10 mL of the urine sample were transferred into a separating funnel and mixed well until the dissolution was complete. The solution was extracted with three 10 mL portions of chloroform, and the organic layer was collected in a beaker after drying over anhydrous sodium sulphate. The solvent was evaporated. The resulting residue was reconstituted with water and diluted to 100 mL with water. The resulting urine solution (100 μg/mL in RIF) was diluted with water to 10, 20, and 30 μg/mL in the FCR method and 20, 30, and 40 μg/mL in the FFC method.

Results and discussion. ***FCR method.*** *Reaction chemistry.* FC is used in the determination of many phenolic compounds [45] and a large number of pharmaceutical substances containing phenolic group [46–57]. The reaction between RIF and the FC reagent in an alkaline medium results in the formation of an intense blue complex with λ_{max} = 760 nm (Fig. 1a). The color formation may be explained similarly to [58, 59]. The coloration of species is probably due to the presence of a reduced heteropoly anion of molybdenum and tungsten [60]. Isopoly and heteropoly anions have been extensively studied, and their structures are fairly well understood. They can be reversibly reduced by the addition of one to six electrons per anion to give the "heteropoly blues", a generic name derived from their intense coloration. These "blues" can be classified as mixed valence complexes containing Mo(V) and Mo(VI) or W(V) and W(VI). It is likely that the phenolic and amino groups of RIF reduce the Mo(VI) and W(VI) centers to produce these relatively stable mixed-valence complexes. The intense fairly broad band observed at 760 nm in the visible/near IR region is due to the presence of these complexes [61]. The large value of ε obtained in this study is consistent with those usually observed for *d-d*-transitions [60, 61].

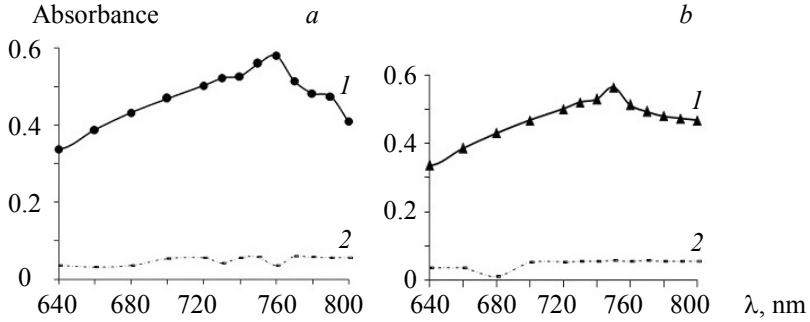


Fig. 1. Absorption spectra: a) RIF-FCR complex (1) (20 μg/mL RIF) for FCR method, b) Fe(II)-PFC complex (1) (30 μg/mL RIF) for FFC method and blanks (2).

Optimization of experimental variables. The experimental conditions were established by varying one variable at a time (OVAT) [62] and observing its effect on the absorbance of the colored species. To find a suitable medium for the reaction, different aqueous materials (such as borax, sodium hydroxide, sodium carbonate, sodium bicarbonate, and sodium acetate) were tested. The best results were obtained when 3 to 5 mL of 20% sodium carbonate solution in a total volume of 10 mL was used; 3 mL was fixed as optimum. Similar observations were made with 3–5 mL of 1:1 FC; 3 mL in a net volume of 10 mL was used as optimal. These results are shown in Fig. 2. Even though color formation was instantaneous, stable readings were produced after 5 min and remained stable for at least 45 min. The order of the addition of reactants followed in the procedure gave the best results.

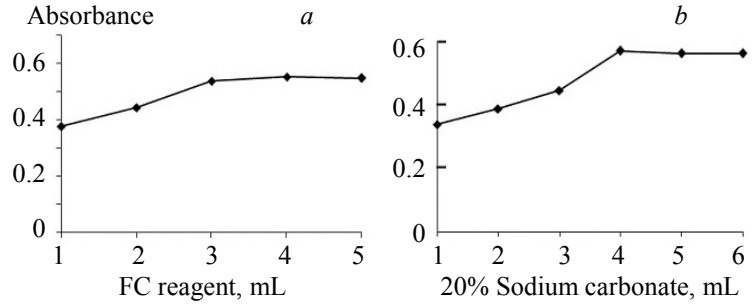


Fig. 2. Effect of FC reagent (a) and alkali concentration (b) (20 μg/mL RIF).

FFC method. *Reaction chemistry.* Iron(III) salts acting oxidants play a very important role in the spectrophotometric assay [63–67]. In this method, iron(III) gets reduced to iron(II) by RIF equivalent to the amount of the drug. The amount of iron(II) formed *in situ* is determined by reacting with ferricyanide. The soluble Prussian blue formed $KFe^{III}[Fe^{II}(CN)_6]$ is measured at 750 nm (Fig. 1b).

Optimization of experimental variables. As shown in Fig. 3, the maximum absorbance is obtained with 0.5 mL of iron(III) solution; larger volumes resulted in decreasing absorbance values. In the case of ferricyanide, 1.5 mL in a total volume of 10 mL was found to produce the maximum absorbance. Based on these observations, 0.5 mL of iron(III) and 1.5 mL of ferricyande solutions were fixed. One milliliter of 0.02 M phosphoric acid was found necessary to arrest the flocculation of the Prussian blue and stabilize the absorbance value. Full color development took 5 min, and it was stable for 2 h thereafter.

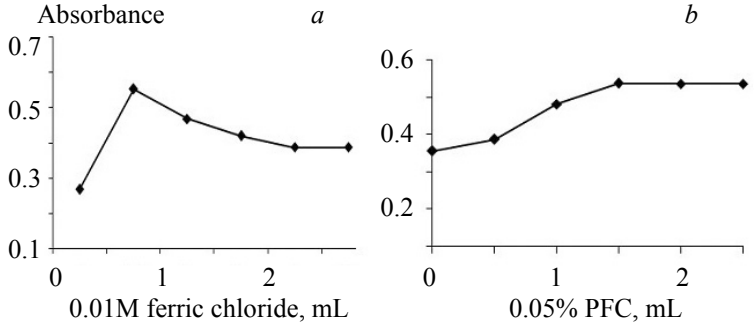


Fig. 3. Effect of 0.01 M ferric chloride (a) and 0.05% PFC (30 μg/mL RIF) (b).

Linearity, sensitivity, limits of detection and quantification. Under the optimum conditions described, Beer's law holds over the concentration ranges 1–35 and 2.5–50 μg/mL for the FCR and FFC methods, respectively (Fig. 4). The graphs are described by the regression equation

$$Y = a + bX,$$

where Y is the absorbance of a 1 cm layer of the solution, a is the intercept, b is the slope, and X is the concentration, μg/mL. Regression analysis of the Beer's law data using the method of the least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) for each system, and the values are presented in Table 1. The optical characteristics, such as Beer's law limits, molar absorptivity and Sandell sensitivity values [68] of both methods, are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) are calculated according to ICH guidelines [68]:

$$\text{LOD} = 3.3S/b, \text{ LOQ} = 10S/b,$$

where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot (Table 1).

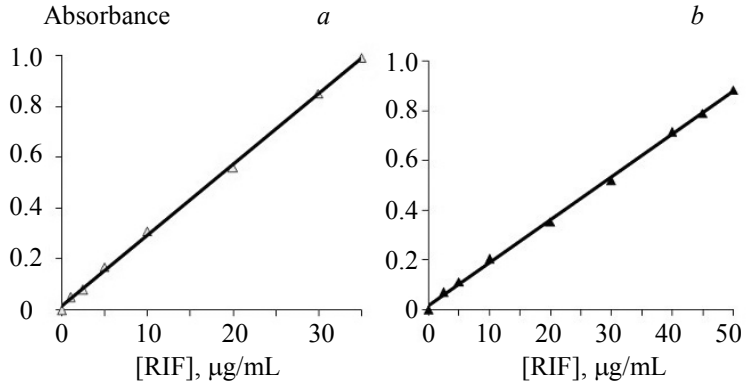


Fig. 4. Calibration plot of FCR (a) and FFC (b) methods.

Precision and accuracy. The procedures described as *general procedures* were repeated 7 times within one day to determine the repeatability (intra-day precision) and 5 times on different days to determine the intermediate precision (inter-day precision) of the methods. These assays were performed at three levels of the analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤1.63% (intra-day) and ≤1.89% (inter-day), indicating high precision. Accuracy

was evaluated as the percentage relative error (RE) between the measured mean concentrations and nominal concentrations for PYP. Bias(%) = $[(C_{found} - C_{known})\times 100/C_{known}]$ was calculated at each concentration, and these results are also presented in Table 2. Percent relative error (%RE) values of <2% demonstrate the high accuracy of the proposed methods.

TABLE 1. Sensitivity and Regression Parameters

Parameter	FCR Method	FFC Method
λ_{max}, nm	760	750
Color stability	45 min	≥ 2 hr
Linear range, μg/mL	1.0-35	2.5-50
Molar absorptivity (ε), L/(mol · cm)	2.72×10^4	1.63×10^4
Sandell sensitivity*, μg/cm^2	0.0302	0.0504
Limit of detection (LOD), μg/mL	0.32	0.32
Limit of quantification (LOQ), μg/mL	0.96	0.98
Regression equation $Y = a + bX$		
Intercept a	0.0224	0.0246
Slope b	0.0276	0.0170
Standard deviation of a (S_a)	1.98×10^{-3}	4.53×10^{-3}
Standard deviation of b (S_b)	2.97×10^{-3}	2.43×10^{-3}
Regression coefficient r	0.9997	0.9996

*Limit of determination as the weight in μg/mL of solution, which corresponds to an absorbance of $A = 0.001$ measured in a cuvette of 1 cm^2 cross-sectional area and $l = 1$ cm.
N o t e. Y is the absorbance, X is concentration in μg/mL.

TABLE 2. Evaluation of Intra-Day and Inter-Day Accuracy and Precision

Method	RIF taken, μg/mL	Intra-day accuracy and precision (n = 7)			Inter-day accuracy and precision (n = 7)		
		RIF found, μg/mL	RSD, %	RE, %	RIF found, μg/mL	RSD, %	RE, %
FCR	10	10.11	0.97	1.10	10.17	1.36	1.70
	20	19.76	1.63	1.20	20.31	0.85	1.55
	30	30.22	1.54	0.74	29.72	1.27	0.93
FFC	20	19.69	0.86	1.55	20.33	1.89	1.65
	30	30.35	0.92	1.17	29.45	1.19	1.83
	40	40.49	1.25	1.22	40.69	0.94	1.72

N o t e. RSD is relative standard deviation, RE is relative error.

Selectivity. The absorbance values obtained for the placebo blank solution were almost equal to the absorbance of the reagent blank, which revealed no interference from the common additives. The analysis of synthetic mixture solution prepared as described earlier yielded average percent recoveries of 98.9±1.86 and 102.1±1.17 (n = 5) for the FCR and FFC method, respectively.

Robustness and ruggedness. The robustness of the methods was evaluated by making small incremental changes in the volume of the reagent, and the effect of the changes was studied on the absorbance of the colored systems. The changes had a negligible influence on the results (RSD ≤2.01%). Ruggedness was demonstrated by the analysis by three analysts, and also by a single analyst performing analysis on three different instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were ≤1.72%. The results are presented in Table 3.

TABLE 3. Method Robustness and Ruggedness Expressed as Intermediate Precision (%RSD)

Method	Robustness		Ruggedness	
	Nominal concentration	Parameters altered	Inter-analysts (n = 3)	Inter-instruments (n = 3)
		Reagent volume* (n = 3)		
FCR	10	1.54	0.85	1.32
	20	1.63	1.27	1.89
	30	2.01	1.16	1.27
FFC	20	1.02	1.72	1.01
	30	1.24	0.81	1.27
	40	1.45	0.92	1.52

* Volumes of reagent varied were 3.0±0.25 mL FC reagent in FCR method and 0.5±0.1 mL of 0.01M $FeCl_3$ in FFC method.

Application to RIF capsules. The proposed methods were successfully applied to the quantification of RIF in capsules. The results were compared with those obtained using the official European Pharmacopoeia method [69]. Capsule extract equivalent to 100 µg/mL RIF was prepared in methanol, 5 mL of this extract was diluted to 10 mL with pH 7.4 phosphate buffer, and the absorbance was measured at 475 nm. The results obtained were compared statistically by the Student's t-test and the variance-ratio F-test. Statistical analysis of the results did not detect any significant difference between the proposed methods and the reference method with respect to accuracy and precision. The results of the assay are given in Table 4.

TABLE 4. Results of Analysis of Capsules by the Proposed Methods

Capsule analyzed	Label claim (mg/capsule)	Official method	Found* (Percent of label claim±SD)	
			FCR Method	FFC Method
R-Cin 300	300	99.5±1.07	101.5±1.29 t = 2.66 F = 1.45	101.2±1.36 t = 2.20 F = 1.62
R-Cin 450	450	101.3±0.95	102.1±1.16 t = 1.19 F = 1.49	102.1±1.35 t = 1.08 F = 2.02

* Mean value of five determinations.
Tabulated t-value at the 95% confidence level is 2.77.
Tabulated F-value at the 95% confidence level is 6.39.

Accuracy by recovery study. The test was performed by spiking the pre-analyzed capsule powder with pure RIF at three different levels (50, 100, and 150% of the content present in the capsule), and the total was found by the proposed methods. Each test was repeated three times, and the recovery values ranged between 98.7 and 101.8% with a standard deviation of 0.86–1.79%. The closeness of the results to 100% showed the fairly good accuracy of the methods. The results are shown in Table 5.

TABLE 5. Results of Recovery Study *via* Standard Addition Method with Capsule

Methods	Formulation studied	RIF in capsule, µg/mL	Pure RIF added, µg/mL	Total found, µg/mL	Pure RIF recovered*, %±SD
FCR	R-Cin 300	10.15	5.0	15.15	100.4±0.97
		10.15	10.0	20.01	99.3±1.79
		10.15	15.0	25.58	101.7±1.05
	R-Cin 450	10.21	5.0	15.39	101.2±1.12
		10.21	10.0	19.95	98.7±1.63
		10.21	15.0	25.66	101.8±0.72
FFC	R-Cin 300	12.15	6.0	18.05	99.2±1.01
		12.15	12.0	24.51	101.5±1.19
		12.15	18.0	30.33	100.6±1.02
	R-Cin 450	12.25	6.0	18.38	100.7±1.25
		12.25	12.0	24.54	101.2±1.04
		12.25	18.0	30.49	100.8±0.86

* Mean value of three determinations.

Application to spiked human urine. The proposed methods were further extended to the RIF assay in spiked human urine samples. The results of the study are summarized in Table 6 and are satisfactorily accurate and precise in the range 94.28–98.36% with a standard deviation of 0.57–1.19%.

TABLE 6. RIF Determination in Spiked Urine Sample, $n = 5$

Method	Spiked concentration, μg/mL	Concentration found, μg/mL	Recovery, %±SD*
FCR	10	9.69	96.86 ±0.91
	20	19.47	97.37±0.57
	30	28.63	95.43±0.72
FFC	20	19.34	96.72±1.07
	30	28.28	94.28±0.69
	40	39.34	98.36±1.19

Conclusion. Two visible spectrophotometric methods for the determination of RIF in bulk drug and in pharmaceutical capsules were developed and validated according to ICH guidelines. The methods employ facile conditions, compared to those used in the previously reported methods, and rely on well-characterized redox-complexation reactions (Table 7). Besides, these methods have the advantages of speed and simplicity without involving heating or extraction; they use aqueous solutions of eco-friendly reagents. The methods can be applied to spiked human urine with good recovery, and no endogenous substances were found to interfere in the assay.

TABLE 7. Performance Characteristics of the Published Visible Spectrophotometric Methods and the Proposed Methods

Sample No	Reagent/reaction	λ_{max}, nm	Linear range (μg/mL), ε (L · mol^{-1} · m^{-1})	Remarks	Reference
1	CT complex formation with DDQ TCNQ *p*-chloranil	 584 680 560	 5–140 5–120 15–200	Less sensitive, organic solvent medium required	[11]
	Complex formation with iron(III)	540	10–240	Less sensitive, requires extraction step and use of organic solvent	
2	Ternary complex formation with pyridine and $La(NO_3)_2$ $NiCl_2$ or $CeCl_3$	 560 580 520	 – 1.45×10^4	Use of expensive chemicals	[12]
3	Redox complexation reaction using NBS-KI-starch	572	0.5–15.5	Less stable NBS used, multi-step reaction	[17]
4	Chelate formation with Cu^{2+} CT complex formation with halogenated quinones	–	40–100	Less sensitive, narrow linear range and use of organic solvent	[36]
5	CT complex formation with *p*-chloranil	500	5–50	Initial pH adjustment required	[37]
6	Redox reaction with ammonium metavanadate	–	–	–	[38]
7	Complex formation with Uranyl acetate Thorium nitrate	 490 525	 –	Expensive reagents required	[39]
8	Redox reaction with DCQC $K_2Cr_2O_7$	 545 540	 0–110 0–210	Less sensitive	[40]

Continue Table 7

Sample No	Reagent/reaction	λ_{max}, nm	Linear range (μg/mL), ε (L · mol^{-1} · m^{-1})	Remarks	Reference
9	Oxidative condensation reaction with PDPD and Chloramine-T	640 520	–	Requires extraction with organic solvent	[41]
10	Ion-pair formation with alizarin violet 3B alizarin brilliant violet R	560	–	Requires critical pH adjustment, extraction step and use of organic solvent	[42]
11	Complex formation with Cu(II) acetate	520	5–30	Requires 20 min standing time and use of organic solvent; narrow linear range and less sensitive	[43]
12	CT complexation with chloranilic acid	–	–	Uses organic solvent medium	[44]
13	FCR FFC	760 750	1.0–35 2.72×10^4 2.5–50 1.63×10^4	Sensitive, wide linear dynamic range, use of eco-friendly inexpensive reagents, use aqueous medium	Present work

* FCR – Folin-Ciocalteau reagent, NBS – N-bromosuccinide.

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