Spectrophotometric Determination of Rifampicin through Chelate Formation and Charge Transfer Complexation in Pharmaceutical Preparation and Biological Fluids

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Two simple and accurate spectrophotometric methods for determination of Rifampicin (RIF) are described. The first method is based on charge transfer (CT) complex formation of the drug with three π-electron acceptors either 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 7,7,7,8-Tetracyanoquinodimethane (TCNQ) or 2,3,5,6-Tetrachloro-1,4-benzoquinone (*p*-chloranil) in acetonitrile. The method is followed spectrophotometrically by measuring the maximum absorbance at 584 nm, 761 nm (680 nm) or 560 nm for DDQ, TCNQ and *p*-chloranil, respectively. Under the optimized experimental conditions, the calibration curves showed a linear relationship over the concentration ranges of 5—140 μg/ml, 2—45 μg/ml (5—120 μg/ml) and 15—200 μg/ml, respectively. The second method is based on the reaction of RIF with iron(III) forming a water insoluble violet complex which is extracted into chloroform. The method determines RIF in concentration range of 10—240 μg/ml at 540 nm. The proposed methods applied to determination of RIF in capsule, human serum and urine samples with good accuracy and precision. The results were compared statistically with the official method and showed no significant different between the methods compared in terms of accuracy and precision.

Key words rifampicin; spectrophotometric determination; charge transfer (CT) complex; π-electron acceptor; iron(III)

Rifampicin, 3-[[4-methyl-1-piperazinyl)-imino]-methyl]-rifamycin SV, (RIF) (Fig. 1a) is a semi-synthetic derivative of Rifamycin SV.[1,2] It can be used alone or in combination with other drugs, such as isoniazid (INH) and pyrazinamide, in treatment of tuberculosis, leprosy and other infectious diseases specially those resulting from AIDS. The high occurrence of tuberculosis in HIV infected subjects makes the management of HIV treatment complex. RIF is a very active antituberculosis drug that accelerates the metabolism of protease inhibitors.[3,4] Due to the increasing necessity to monitor plasma concentrations in HIV patients with tuberculosis, different methods such as spectroscopy,[5—9] fluorometry,[10—12] gas chromatography,[13] polarography,[14] amperometry[15] and high performance liquid chromatography[16—21] have been developed to measure RIF alone or in the presence of INH. These methods are expensive and need more expertise in experimentation in comparison with spectrophotometry.

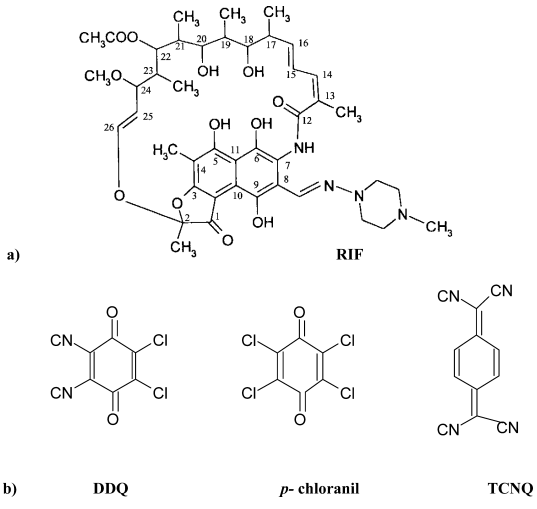

Fig. 1. Rifampicin and π-Electron Acceptors Structures

Spectrohotometric techniques continue to be the most preferred method for routine analytical work due to their simplicity and reasonable sensitivity with significant economical advantages. On the other hand, it is well known that *p*-benzoquinones such as 7,7,7,8-tetracyanoquinodimethane (TCNQ), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 2,3,5,6-Tetrachloro-1,4-benzoquinone (*p*-chloranil) as π-electron acceptors (Fig. 1b) often form highly colored electron-donor–acceptor (EDA) or charge transfer (CT) complexes with various donors which provides the possibility of determination of drugs by spectrophotometric methods.

In order to develop a new method for determination of RIF in pure and pharmaceutical forms as well as characterize the behavior of RIF in EDA chemistry, we investigated spectrophotometrically the reactions of RIF with organic π-acceptors DDQ, TCNQ and *p*-chloranil. The absence of an extraction step in these assays and the efficiency of the method offer the ease and rapidity desired for measurement of RIF. Also, an extractive-spectrophotometric method based on the reaction of RIF with iron(III) was developed. The results were compared with other similar methods. The reaction pathways were investigated by IR and ^{1}H-NMR spectra.

Results and Discussion

RIF was found to yield intense colors with π-acceptors in acetonitrile solution, most probably due to the CT complexation of RIF as *n*-electron donor with DDQ, TCNQ and *p*-chloranil as π-electron acceptors. Figure 2 shows the absorption spectra of RIF, DDQ, TCNQ and *p*-chloranil and their complexes in acetonitrile. Acetonitrile was found to be an ideal solvent for the formation of charge transfer (CT) complex. Methanol, dichloromethane, chloroform and mixtures of methanol-acetonitrile were examined and produced lower absorbance reading. Acetonitrile was chosen as the best solvent due to intensely colored CT complex with high molar absorptivity values and more reproducibility of the measure-

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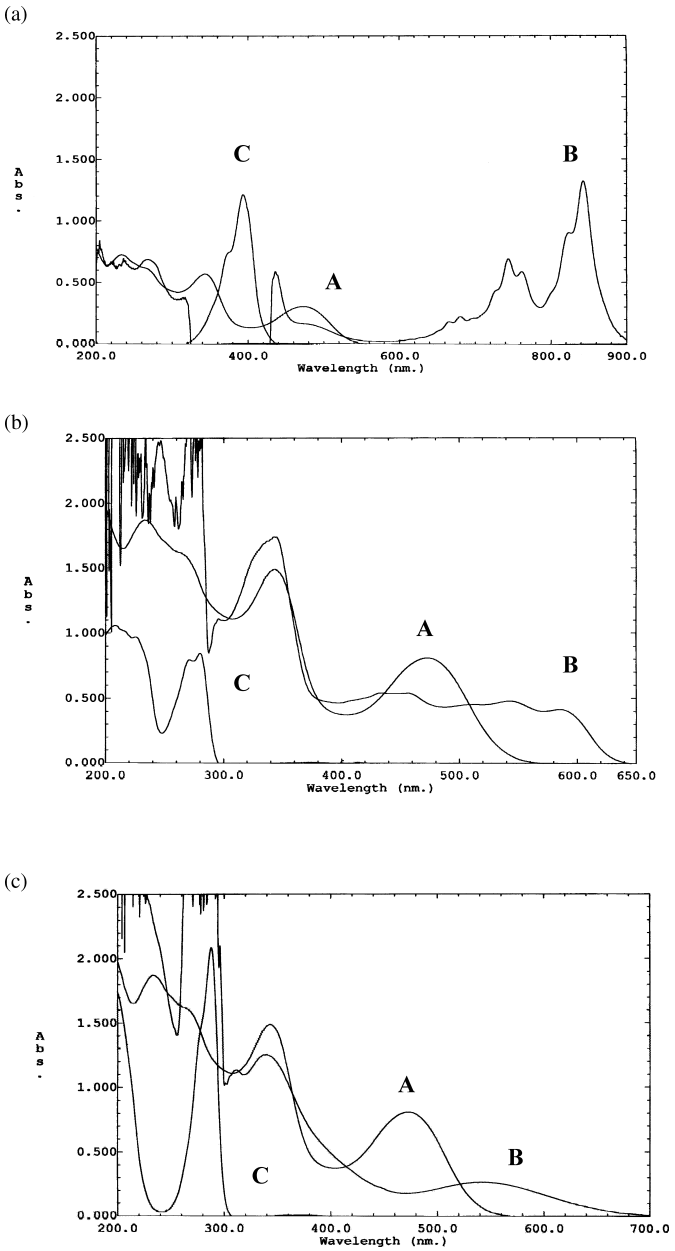

Fig. 2. Absorption Spectra of (A) RIF and (B) RIF Complexes with (C): (a) TCNQ (0.01% (w/v)); (b) DDQ (0.01% (w/v)) and (c) *p*-Chloranil (0.016% (w/v)) in Acetonitrile

[RIF] = 20 μg ml^{-1}.

ments.

TCNQ is a strong π-electron acceptor to react with RIF, producing green bluish product. As seen in Fig. 2a, while none of the reactants showed any considerable absorption in the 600—900 nm range, addition of the drug to the TCNQ solution resulted in new absorption bands with maxima at 842, 761, 680 and 665 nm, presumably due to the formation of a CT complex, so the overall spectral feature of TCNQ greatly was changed. 761 and 680 nm were selected for further quantitative work due to stability of absorbance and reproducibility of the measurements. The reaction was studied as a function of the concentration and volume of the reagent. Stability of color of CT complex and the molar ratio of the reactants were also studied. As shown in Fig. 3, the absorbance increased with increasing the concentration of

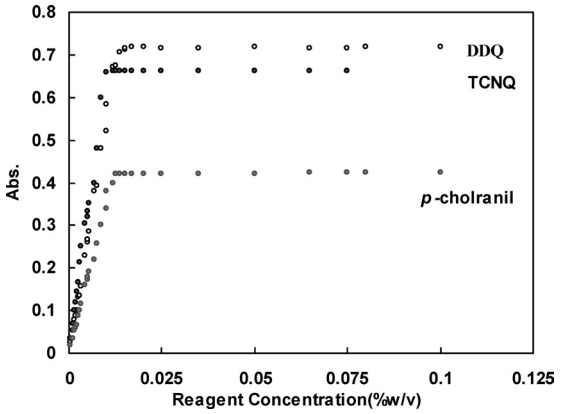

Fig. 3. Effect of Reagent Concentration (A) TCNQ; (B) DDQ; (C) *p*-Chloranil on the Formation of Complexes with RIF

RIF concentrations are 70 μg ml^{-1}, 90 μg ml^{-1} and 80 μg ml^{-1}, respectively.

TCNQ until a plateau was reached. The obtained data showed that 1 ml of 0.05% (w/v) reagent solution was efficient for quantitative measurement so higher reagent concentration didn't affect the color intensity.

DDQ is a strong π-acceptor but weaker than TCNQ.[22] Interaction of DDQ with RIF formed a purple-red color product in acetonitrile with absorption maxima at 460, 546 and 584 nm (Fig. 2b). Such spectral features are in agreement with those reported before for $DDQ^{\cdot-}$ radical ion formation[23,24] which is shown that transfer of π-electron from donor to acceptor moiety in polar medium (acetonitrile) through $DDQ^{\cdot-}$ radical formation has occurred. Measurements were carried out at 584 nm which was far from the maximum wavelength of RIF (475 nm). Variation of concentration and volume of the reagent indicated that 1 ml of DDQ at concentration of 0.05% (w/v) was sufficient to reach a stable absorbance. Investigation of time on the absorbance showed that after 30 min, the absorbance of the CT complex remained constant for 3 h. The application of Job and mole ratio methods[25] indicated 1 : 1 mole ratio for the investigated CT complex of RIF with DDQ.

By evaluating the UV–Vis. spectrum of reaction of RIF with *p*-chloranil (Fig. 3c), it was found that complexity of the spectrum was similar to mono substituted amino-quinones. It showed new broad band around 560 nm which was used for quantitative work. The weak and small absorptivity of this band may be explained on the lower electron affinity of *p*-chloranil. One millilitor of 0.08% (w/v) of *p*-chloranil solution was found to reach maximum absorbance reading at the corresponding maximum after 5 min and the absorbance was stable for 24 h. Evaluation of stoichiometry of CT complex indicated a 1 : 1 RIF/*p*-chloranil ratio.

Under the optimum experimental conditions obtained for the CT complexes, the relationship between the concentration and the absorbance of the color formed using *p*-chloranil, DDQ and TCNQ were determined. Beer's law is obeyed in the concentration ranges of 15—200 μg ml^{-1}, 5—1400 μg ml^{-1}, 5—120 μg ml^{-1} (at 680 nm), and 2—45 μg ml^{-1} (at 761 nm) using *p*-chloranil, DDQ and TCNQ respectively, as shown in Table 1. Also, Table 1 illustrates regression equations, correlation coefficients (*r*) and Sandell's sensitivity for the proposed methods. Limit of detection was obtained as the sample concentration that produces an ab-

Table 1. Quantitative Parameters for the Reaction of RIF with π-Acceptors and Fe(III) Ion

Parameters	TCNQ λ_{max}=761 nm λ_{max}=680 nm	DDQ λ_{max}=584 nm	*p*-Chloranil λ_{max}=560 nm	Fe λ_{max}=540 nm	Ref. 26 λ_{max}=500 nm	Ref. 30 λ_{max}=560 nm	Ref. 7 λ_{max}=562 nm
Linear range; μg ml^{-1}	2—45 5—120	5—140	15—200	10—240	5—50	40—200	40—100
Absorptivity coefficient; 1 mol^{-1} cm^{-1}	2.84×10^4 6.50×10^5	6.58×10^5	4.11×10^5	4.06×10^5	9.80×10^5	—	—
Regression equation	Y=0.0265X +0.0088 Y=0.0080X +0.1108	Y=0.0080X +0.0025	Y=0.0050X +0.00105	Y=0.0049X −0.0095		—	Y=0.0078X −0.0081
Correlation coefficient (r)	0.9999 0.9948	0.9941	0.9983	0.9999		—	0.9998
Detection limit; μg ml^{-1}	0.90 2.09	2.59	3.95	2.30		—	10
Sandel sersitivity, ng cm^{-1}/0.001A	37.0 126.0	125.0	200.0	202.4	84.0	570.0	—
%RSD	1.10 0.28	0.37—1.56	0.42—1.76	0.59—1.78	0.96	0.22	0.82
Stability of calor (h)	2 3	3	24	24	1.5	—	NM

NM=not mentioned.

sorbance with three times the blank standard deviation.

The selectivity of the method was tested by examining the possible interference from isoniazide (INH), which might be concomitantly administered with RIF, and hydrolyzed products of RIF as mentioned in the experimental section. Only in *p*-chloranil method, spectral interference of the hydrolyzed product in reaction with *p*-chloranil was observed. INH had no interference in determination of RIF except in DDQ method. This problem could be solved by one extraction step before treatment with DDQ.

Recent studies showed[27] that RIF could interact with Cu(II), Co(II), Al(III), Ni(II), Ce(III), La(III) or Zr(IV) ions in methanol giving a colored chromogen to quantitative determination of RIF. Beers' law was obeyed in the ranges of 40—100 μg ml^{-1} by cupric ion and 2—70 μg ml^{-1} by the other metals. INH also reacted with the cupric ion similarly as RIF and showed interference. RIF was found to react with ferric ion to give a violet color complex insoluble in water. The absorption spectrum of the reaction product exhibited a maximum absorbance at 540 nm in chloroform (Fig. 4). The stability of RIF–Fe(III) chelate (K_f=5.0×10^8) in chloroform indicates the high stability of the chelate. The chelate formation was studied as a function of pH. Absorbance of the product was constant using aqueous solution at pH<2.0. In alkaline media, hydrolysis of iron(III) occurs. The effect of iron(III) concentration on the intensity of the color developed at the corresponding wavelength was ascertained by changing the volume and concentration of Fe(III) solution when ferric ion was added to a fixed concentration of RIF. The results showed that maximum absorbance was obtained using 2 ml of 0.3% (w/v) ferric ion solution. The optimum conditions for the reaction between ferric ion and RIF was found 2 ml of 0.3% (w/v) ferric ion solution at pH 2.0, 6 ml aqueous solution and 5 ml of chloroform. More investigation showed that counter ion and ionic strength had no effect on the color intensity of the extracted product. The absorbance of the complex remained constant for at least 24 h at room temperature. Beer's law was obeyed over the concentration range of 10—240 μg/ml. The results are summarized in Table 1. INH had no interference on determination of RIF.

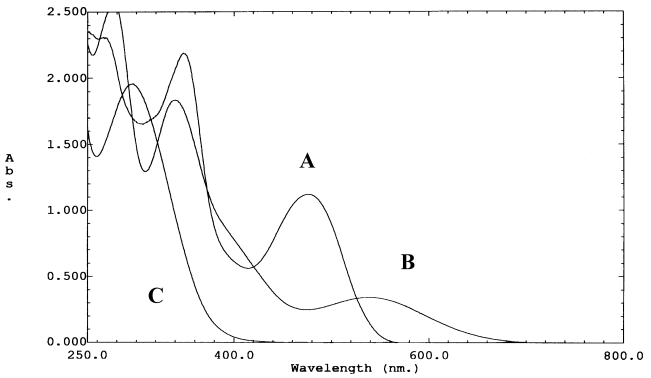

Fig. 4. Absorption Spectra of (A) RIF, (B) RIF–Fe(III) Complex in $CHCl_3$ and (C) Fe(III) Ion in Aqueous Solution
[RIF]=50 μg ml^{-1}.

The crystalline solids of RIF's complexes with different π-electron acceptors were prepared as described in the experimental section. The formation of CT complexes was confirmed by both IR and ^{1}H-NMR techniques. The IR spectra of the RIF, π-electron acceptors and isolated complexes in the wavenumber range 4000—400 cm^{-1} were recorded. The resulting spectral data with the tentative assignments of the most important IR frequencies of the molecular complexes, π acceptors[28—30] and RIF are summarized in Table 2. From the data are given in the Table 2, decreases in the vibration frequencies of a particular band have been used as evidence for a particular site of a CT interaction. It is clear that most of the fundamental frequencies of π acceptors show significant shifts to lower wavelength, strongly supporting the formation of the RIF-π acceptors CT complexes. For example, ν(C=O) and ν(azomethine) in RIF showed 10 and 30 cm^{-1} shift, respectively, to lower frequencies upon complexation with TCNQ, most probably indicating the direct participation of nitrogen atom in the molecular complex formation process. Meanwhile, ν(C–CN) in TCNQ showed a relatively large shift, 40 cm^{-1}, to higher frequency as a result of complexation with RIF and the band in donor is splitted. This splitting

Table 2. IR Spectral Data of RIF, π-Acceptors and their 1 : 1 Molecular Complexes

Assignment cm^{-1}	TCNQ	DDQ	*p*-Chloranil	RIF	TCNQ–RIF	DDQ–RIF	*p*-Chloranil–RIF	Fe–RIF
ν (C–N)	2220	2227	—	—	2180	2220	—	—
ν (C=C–CN)	1540	—	—	—	1570	—	—	—
ν (C–Cl)	—	800	710, 750	—	—	Absent	710	—
ν (C=O)	—	1675	1680, 1690 (s)	1640	1630 (br)	1630	1630	1630
ν (azomethine)	—	—	—	1650	1600 (br)	1620 (w)	1620	1620
ν (C–O)	—	—	—	1240	1240	1240	1240	1250
ν (N–H) deformation	—	—	—	1550	Absent	Absent	1520 (w)	1520 (w)

means the symmetry of the cyano groups in TCNQ is lost due to interaction with RIF. Similar displacement of IR frequencies of DDQ and *p*-chloranil could be seen in their complexes.

The ^{1}H-NMR spectra of RIF and RIF–DDQ were recorded in $CDCl_3$ solution and the related spectra are illustrated in Fig. 5. It is characterized by one sharp singlet at 3.0 ppm for the OCH_3 protons on carbon 24, sharp singlet signal at 8.21 ppm can be attributed to the azomethine proton on carbon 8 and two broad singlets, one at 12.04 (phenolic protons) and another at 13.22 ppm (N–H proton). Both the latter hydrogens are exchangeable by addition of D_2O. The most acidic proton is the proton on carbon 9 as OH group so by deprotonation of the hydroxyl group and formation a –C=O function, the resonance in the benzene ring is lost and charge density of the nitrogen atom of azomethine group reaches to a maximum. The absence of –NH signal indicates the participation of the amide group in this reaction. Charge transfer from the RIF to DDQ probably occurred through nitrogen atoms of amide and azomethine groups in RIF. Moreover, methylene protons at carbon 4 and 9 gave NMR signals at 2.6—3.0 ppm while methine protons at carbon 14, 15, 16, 25, and 26 gave a multiplet at 5.0—6.45 ppm. In CT complex, these signals were shifted to 2.4—3.0 ppm and 5.09—6.81 ppm, respectively. While methyl protons on carbon 21 were shifted to a lower field in CT complex (from −0.27 ppm to 0.11 ppm), a slightly lower field of methyl protons on carbon 19, 17 and 13 is also noticed (from 1.06, 0.64 and 1.80 to 1.05, 0.61 and 1.82 ppm). The similar spectrum was obtained in the case of Fe–RIF complex.

Accuracy of the proposed methods was investigated by recovery studies. The proposed methods were successfully applied to the analysis of RIF in 150 and 300 mg capsules of two different commercial companies by standard addition technique. The results of the assay by the proposed methods are presented in Table 3. The excellent recoveries indicate the absence of interference from frequently encountered excipients or additives in RIF capsules such as magnesium stearate, titanium dioxide, gelatin, starch and colors FD&C Blue No. 1, D&C Red No. 28 and FD& Red No. 40. The performance of the methods was evaluated by calculation of the *t*- and *F*-values compared with the official method according to the BP.[1)] Mean values were obtained in *t*- and *F*-tests at 95% confidence limits for 8 degrees of freedom. The results showed that the calculated *t*- and *F*-values didn't exceed the theoretical values. This means there is no significant difference between the proposed and the official methods. The accuracy of the methods was evaluated by spiking the known amounts of the drug to biological fluids such as urine and human serum. The obtained recoveries were in the range of 99.1—99.6% (n=3).

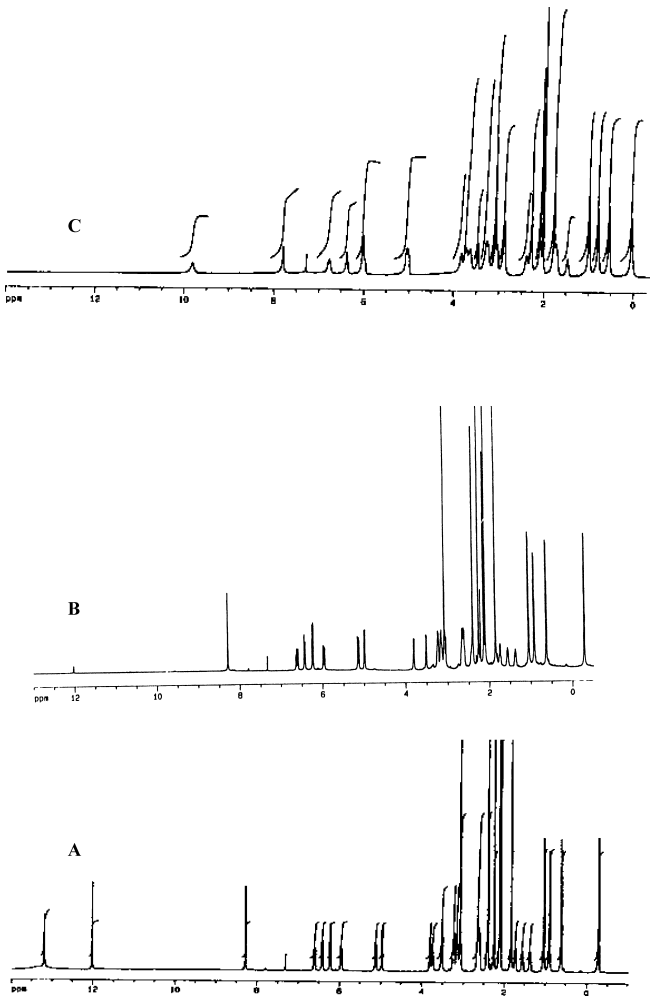

Fig. 5. ^{1}H-NMR Spectrum of (A) RIF in $CDCl_3$ (B) RIF–DDQ Complex in D_2O and (C) RIF–DDQ Complex in $CDCl_3$

The proposed method based on *p*-chloranil was compared with the previous reports on reaction of RIF with *p*-chloranil in methanol at 500 nm.[7,26)] The result of the chelate method was also compared with the spectrophotometric method based on extraction-spectrophotometry determination of RIF with methavanadate in acidic solution[31)] or direct determination of RIF with cupric ion in methanol.[7)] The advantages of the proposed methods are high stability of the products and wide working concentration ranges and low detection limit.

Conclusion

The proposed spectrophotometric methods have the advantage of being sensitive and simple for routine analysis of RIF,

Table 3. Statistical Comparison between Results of Analysis of RIF in Capsule, Urine and Serum Samples Using the Proposed and Official Methods

Sample	Labeled amount (mg)	TCNQ (λ_{max}=761 nm)	DDQ (λ_{max}=584 nm)	*p*-Chloranil (λ_{max}=560 nm)	Fe (λ_{max}=540 nm)	Found by official method
Capsule (Alhavy)	150					147.2±1.1
Found		149.2±1.8	149.6±2.0	149.3±1.8	149.6±1.5	
t-value		0.63	0.70	0.30	0.75	
F-value		2.68	3.30	2.68	2.10	
Recovery %		99.4±1.2	99.7±1.3	99.5±1.2	99.6±0.7	
Capsule (Hakim)	150					149.0±1.4
Found		149.8±1.9	149.6±2.0	150.0±1.6	149.4±1.0	
t-value		0.22	0.27	1.50	0.15	
F-value		1.84	2.04	1.31	1.96	
Recovery %		99.9±1.2	99.7±1.3	100.0±11	99.6±0.7	
Capsule (Hakim)	300					297.3±2.2
Found		300.6±1.8	299.5±2.1	299.3±1.2	299.2±2.2	
t-value		1.04	0.59	0.53	0.34	
F-value		1.49	1.10	3.36	1.00	
Recovery %		100.2±0.6	99.9±0.7	99.8±0.6	99.7±0.8	
Urine	Spiked: 5(20) μg/ml	4.98±0.06	4.95±0.50	19.79±0.31	19.91±0.30	
Serum	Spiked: 5(20) μg/ml	4.96±0.10	4.94±0.15	19.80±0.42	19.90±0.30	

p=0.05; theoretical *F*- and *t*-values: 4.43, 2.12; n_1=n_2=9; in serum and urine samples, the spiked concentration was 20 μg/ml.

although the method based on complexation of RIF with iron(III) needs a one step extraction. The TCNQ acceptor was more sensitive than the others due to higher molar absorptivity. TCNQ and DDQ methods are faster than the *p*-chloranil method. The color of CT complex of *p*-chloranil with RIF was more stable than the color of complex with TCNQ and DDQ methods. No significant differences between the proposed and official methods were obtained. The developed methods are suitable for the analysis of RIF in pure and capsule forms in control laboratories. Also, these methods can be used to determine RIF in biological fluids of patients.

Experimental

Reagents and Materials All chemicals and solvents were purchased from Merck and used without more purification. INH was obtained in analytical grade from Food and Drug Quality Control Laboratory, Ministry of Health and Medical Education, Tehran, Iran. Pure RIF was gift sample from commercial source (Hakim Pharmaceutical Company, Tehran, Iran) and was used without further purification.

Stock solution of RIF with concentration of 500 μg/ml was prepared in acetonitrile. DDQ and TCNQ solutions were prepared at 0.05% (w/v) and *p*-chloranil at 0.08% (w/v) concentration in acetonitrile. $FeCl_3 \cdot 6H_2O$ was prepared at 0.1% (w/v) concentration in double distilled water.

Procedure. General Procedure for CT Complex Method DDQ Method: Suitable aliquot of the drug stock solution containing 5—140 μg/ml was pipetted into a 5-ml volumetric flask. One milliliter of DDQ solution was added and diluted to volume with acetonitrile and mixed thoroughly. The mixture was allowed to stand for about 0.5 h at room temperature and then the absorbance of the purple-red solution was measured at 584 nm (Shimadzu UV–Vis. double beam spectrophotometer Model 2501) against the reagent blank prepared in the same manner.

TCNQ Method: Standard solution containing known quantities of RIF were put in a 5 ml volumetric flask and 1 ml of TCNQ solution was added. The solution was diluted to volume with acetonitrile and allows the mixture to stand at ambient temperature for about 1.5 h. The absorbance of the green-blue solution was measured at 761 and 680 nm against a reagent blank similarly prepared.

p-Chloranil Method: An accurate volume of stock solution of RIF was transferred into a 5 ml volumetric flask. One milliliter of *p*-chloranil solution was added and diluted to volume with acetonitrile. The absorbance of the purple solution was measured instantly at 560 nm against the reagent blank.

Procedure for Chelating Method A stock solution containing 500 ppm of RIF in $CHCl_3$ was prepared. $FeCl_3 \cdot 6H_2O$ 0.3% (w/v) solution freshly was prepared. To a 125 ml separating funnel, 2 ml of $FeCl_3 \cdot 6H_2O$ 0.3% (w/v), 5 ml of diluted RIF in $CHCl_3$ and 4 ml distilled water were added. The mixture was shaken for 7 min and the organic layers were collected and filtered through anhydrous sodium sulfate into a 10 ml volumetric flask. The absorbance of the violet solution was measured at 560 nm against a reagent blank similarly prepared.

Procedure for Determination of RIF in Capsules Ten capsules was weighed accurately and powdered in a mortar. An amount of the finely powdered capsules equivalent to 5 mg of the drug was dissolved in acetonitrile and sonicated for 5 min and then was filtered. The further dilution of the extract was made with the same solvent into a 10 ml volumetric flask to obtain a final solution containing 500 μg/ml of RIF. Then 0.4 ml of the resulting solution was transferred to a 5 ml volumetric flask and processed as authentic drug by standard addition method. In chelating method, preparation of the stock solution of capsules was the same as described in the assay procedure but the powder was dissolved in chloroform. 0.8 ml of this solution was taken and processed as described under "procedure" section. The amount of RIF in capsule was obtained by standard addition method.

Procedure for Determination of RIF in Urine and Human Serum Elimination of nonpolar urine constituents was accomplished by extraction using chloroform. A 1 ml aliquot of urine sample was spiked with pure RIF solution and was diluted with distilled water to obtain a solution containing 5 μg/ml of RIF (in the case of *p*-chloranil and chelate method, the concentration was 20 μg/ml). This solution was transferred to a separating funnel and the contents extracted with 3×10 ml quantities of chloroform. The chloroform fractions were collected and evaporated to dryness. The residue was dissolved in acetonitrile and 1 ml of CT reagent was added and further diluted with the same solvent to 5 ml in a volumetric flask. The recommended procedures for measuring of RIF were followed. The same procedure was followed by the reagent blank samples. In chelate method, the residue was dissolved in chloroform.

In the case of serum, first stage was extraction for deproteinization of serum, for which 2 ml acetonitrile was added to chloroform. Remaining procedure was the same as described above for urine sample. The mean recovery of the drug in urine and human samples were obtained from three replicate measurements and summarized in Table 4.

Determination of RIF in the Presence of Its Hydrolysis Product and Isoniazid Rifampicin was refluxed in the presence of hydrochloric acid. The products were 3-formyl rifamycin SV and 1-amino-4-methyl pipirazine. The latter was soluble in water and separated from the former. When hydrolysis process completed, the obtained solution was neutralized with NaOH; the precipitate was washed with water and dried in vacuum desiccators under P_2O_5. The products were identified with melting point, IR (Model 781, Shimadzu) and ^{1}H-NMR Spectra (Bruker Model DRX-500 Avance). For determination of RIF in the presence of insoluble hydrolyzed product in water, 0.5 ml of 500 μg/ml solution of RIF and 0.5 ml of 500 μg/ml of hydrolyzed product solution in acetonitrile were transferred into a 5 ml volumetric flask. One milliliter of CT reagent was added and diluted to volume with the solvent. The absorbance of the colored solution was measured at corresponding wavelength against the blank similarly treated. The procedure for chelating

method was the same as described before. For determination of RIF in the presence of INH as interferant, in another experiment an almost equivalent amounts of RIF and INH (0.2 ml of 500 μg/ml) were transferred to a 5 ml volumetric flask and the procedure was followed as described in the "procedure" section for both methods.

Preparation of Solid Products For preparation of solid products in reaction of RIF with π-electron acceptors, a solution of each reactant at concentration 1.0×10^{-3} mol/ml in acetonitrile was mixed with RIF solution with the same concentration and the solvent was allowed to evaporate. Then the residue was dissolved in chloroform and mixed with water in a separating funnel to ensure that HCN or HCl which is produced in the reaction of RIF with DDQ or *p*-chloranil, could extract into the aqueous phase. After separating the two phases and removing the probable droplet of water in chloroform phase with anhydrous sodium sulphate, the solvent was evaporated and the solid product was dried in a vacuum desiccator under P_2O_5. Uncorrected melting point of each product was examined. IR absorption spectra of the molecule complexes were measured at room temperature by use of KBr pellets with IR spectrophotomer, Shimadzu Model 781. ^{1}H-NMR spectra of the complexed and free compounds were recorded by Bruker Model DRX-500 Avance.

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