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Redox and Complex Formation Reaction–Based Spectrophotometric Assay of Rifampicin in Pharmaceuticals and Urine

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KEYWORDS

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Capsules,
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ABSTRACT:

Introduction: Rifampicin (RIF), 3-[(4-methyl-1-piperazinyl) imino methyl] rifamycin SV [Figure 1], is a semi synthetic bactericidal antibiotic, active *in vitro* against gram-positive microorganisms and mycobacteria [1]. It is used in the treatment of tuberculosis and other infections and widely applied in human and veterinary medicine. There is drug toxicity during the treatment with RIF, especially in patients with human immunodeficiency virus (HIV) infection, and there has been a global increase in the prevalence of drug-resistant tuberculosis. Therefore, drug monitoring in patients during anti-TB therapy is important in the case of drug resistance and toxicity [2,3].

Objectives: Two rapid, simple, sensitive and selective spectrophotometric methods were developed and validated for the assay of rifampicin (RIF) in pure form, capsules and spiked human urine.

Methods: The methods were based on the reduction of iron(III) chloride by RIF and subsequent chelation of iron(II) with 1,10-phenanthroline (Phen method) and 2,2'-bipyridyl (Bipy method). The absorbances of resulting colored products were measured at 520 and 540 nm, respectively. Experimental conditions for the assay were optimized.

Results: Regression analysis of Beer's law plots showed good correlation in the concentration ranges, 2.5-45 and 2.5-50 µg mL^{-1} with apparent molar absorptivities of 1.91×10^4 and 1.68×10^4 L mol^{-1} cm^{-1} for Phen method and Bipy method, respectively. The Sandell sensitivity values, limits of detection (LOD) and quantification (LOQ) values have also been reported for both methods. The accuracy and precision of the methods were evaluated on between-day and day-day basis; the relative error (%RE) was ≤ 2.36% and the relative standard deviation (RSD) was ≤ 1.94%. The developed methods were successfully applied to the determination of drug in RIF capsules and spiked human urine with good recovery. Statistical comparison of the results with the reference method showed good agreement and indicated no significant difference in accuracy and precision.

Conclusions: Two visible spectrophotometric methods for the assay of RIF in drug substance, drug product and in spiked human urine were developed and validated for accuracy, precision, linearity, robustness and ruggedness. The methods employ normal conditions compared to those previously reported, and rely on well-characterized redox-complexation reactions (chelation). Besides, these methods have the advantages of simplicity without involving heating or extraction step; use aqueous solutions of eco-friendly reagents. When extraction difficulties arise with other published methods (Table 7), with these methods, one can do the analysis at low cost without losing accuracy. The methods can be used as an alternative method to reported ones for the routine determination of RIF in drug substance, drug product and in spiked human urine.

1. Introduction

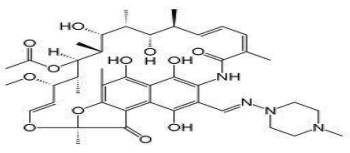

Figure 1. Chemical structure of rifampicin [RIF]

Several methods are available for the determination of RIF in biological fluid samples including visible spectrophotometry [4-9], fluorimetry and microbiology [10], cyclic and square wave voltammetry [11], nuclear magnetic resonance spectrometry [12] and high performance liquid chromatography (HPLC) [13,14].

Simultaneous determination of RIF and other antituberculosis drugs in pharmaceutical dosage forms has been accomplished

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using such techniques uv-spectrophotometry and its other variances like multivariate [15-17], first-derivative [18-20], double-divisor ratio [21], graphical absorbance ratio and absorbance additive [18] spectrophotometry, cyclic and square wave voltammetry [11], linear sweep and cyclic voltammetry [22], differential pulse polarography [23,24], horseradish peroxidase (HRP)-based amperometry [25], chemiluminescence spectrometry [26,27], HPLC [13,28,29], and HPTLC [30,31].

Literature mentions only a few methods such as direct uv-spectrophotometry [9], nuclear magnetic resonance spectrometry [12], chemiluminescence spectrometry [27], HPLC [32] and differential pulse polarography [33,34]. Although visible spectrophotometry is the instrumental technique of choice commonly used in industrial laboratories, for its simplicity, selectivity and sensitivity, the technique has not been widely used for the assay of RIF.

Sastry*et al.* [4] have described two methods by measuring the absorbance of complexes formed with VO_2^+ and Th(IV) in the presence of PrOH at 490 and 525 nm, respectively. Cu(II) was found to form a colored complex with RIF in methanolic medium paving the way for the assay of drug [5]. The drug was determined by Sadegi and Karimi [6] through complex formation with iron(III) and charge-transfer complexation with three pi-electron acceptors in acetonitrile medium. Sastry*et al.* [7] used the route of ternary complex formation reactions involving RIF & $ZrOCl_2$, $La(NO_3)_2$, $NiCl_2$, or $CeCl_3$in the presence of pyridine in methanolic medium. Ion-pair extraction assay [8] using either alizarin violet 3B or alizarin brilliant violet R and measuring the absorbance of the colored complex at 560 has been reported by Reddy *et al.* Complex formation with Cu(II) and C-T complexation reaction with halogenated quinones were used by Shereen*et al.* [35] for the assay of RIF.

Halogenated quinones such as chloranil [36] and chloranilic acid [37] were also used as chromogenic agents through C-T complexation reaction for RIF. Shukla*et al.* [38] developed a method by reacting RIF with ammonioummetavanadate in acid medium. In an indirect method reported by Barsoum*et al.* [9], the drug was reacted with a known excess of N-bromosuccinimide and the unreacted oxidant was treated with KI and the resulting trioxide ion $I_3^-$was measured. Two spectrophotometric methods [39] based on reactions with 2,6-dichloroquinone chlorimide and $K_2Cr_2O_7$ with RIF are also found in the literature. In a method reported by Diwakar*et al.* [40], the drug was reacted with p-N,N-dimethylphenylenediamine and chloramine-T, and the resulting chromogen was extracted into butyl alcohol and absorbance measurement at 520 nm.

The above methods [4-9, 35-40], are deficient is one way or the other and are less sensitive with narrow linear dynamic range, require strict pH control, extraction step, longer contact time and\or use of organic solvent as reaction medium.

This paper describes two spectrophotometric methods based on the reducing property of RIF wherein iron(III) is reduced to iron(II) which is chelated with either 1,10-phenanthroline and absorbance measured at 520 (Phen method) or 2,2'-bipyridyl and absorbance measured at 540 nm (Bipy method); these reactions have successfully been used to quantify many drugs [41-47] previously. The proposed methods were found to be superior to many published methods in several aspects.

2. Objectives

Experimental

Apparatus

All spectral runs and absorbance measurements were made using a Systronics model 166 digital spectrophotometer (Ahmedabad, India) equipped with 1 cm matched quartz cells.

Reagents and materials

All Chemicals and reagents used were of analytical reagent grade or chemically pure grade and used without further purification, and double distilled water was used throughout the investigation.

Ferric chloride ($FeCl_3$) (0.01M): The aqueous solution of 0.1M $FeCl_3.6H_2O$ (S.D. Fine Chem., Mumbai, India) was prepared by dissolving 2.7 g of the chemical in 100 mL of 0.1M HCl. The resulting solution was diluted to get 0.01M $FeCl_3$ with water and used in both methods.

*1,10-phenanthroline (phen) (0.01M):*The solution was prepared by dissolving 198 mg of the chemical (Qualigens Fine Chemicals, Mumbai, India, assay 100%) in water and diluted to 100 mL.

*2,2'-Bipyridyl (bipy) (0.01M):*The solution was prepared by dissolving 156 mg of the chemical (Qualigens Fine Chemicals, Mumbai, India, assay 100%) in water and diluted to volume in a 100 mL calibrated flask.

*Standard drug solution:*Pharmaceutical grade RIF (99.9% purity) was a gift from Lupin Limited, Tarapur, Maharashtra, India, and was used as received. Capsules in two strengths R-Cin 300 and R-Cin 450 capsules (Lupin Limited, Chikaltana, Aurangabad, India) were purchased from local commercial stores.

A stock solution containing 100 µg mL^{-1} rifampicin was prepared by dissolving 10 mg of the drug in 100 mL water in a calibrated flask.

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3. Methods

Assay procedures

Preparation of calibration graph

Phen method

Varying aliquots (0.0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 4.5 mL) of standard RIF solution (100 µg mL^{-1}) were accurately measured into a series of 10 mL standard flasks by means of a micro burette and the total volume was brought to 4.5 mL by adding water. To each flask added 1.0 mL each of 0.02M orthophosphoric acid and 0.01M $FeCl_3$ followed by 2.0 mL of 1,10-phenanthroline. The content was mixed well and diluted to the mark with distilled water. The absorbance of each solution was measured at 520 nm against reagent blank after 10 min.

Bipy method

Different aliquots (0.25–5.0 mL) of standard RIF solution (100 µg mL^{-1}) were transferred into a series of 10 mL volumetric flasks using a micro burette and the total volume was adjusted to 5.0 mL with water. To each flask, 1.0 mL each of 0.02M orthophosphoric acid and 0.01M $FeCl_3$ and 2.0 mL of 0.01M 2,2'-bipyridyl 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 red coloredchromogen was measured against the reagent blank at 540 nm.

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

Procedure for capsules

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 50 mL of water for 20 minutes and the content was diluted to the mark with water. It was filtered using Whatman No 42 filter paper. First 10 mL portion of the filtrate was discarded and 3.0 mL portion of the subsequent portion was subjected to analysis following the assay procedures described earlier.

Procedure for selectivity by placebo and synthetic mixture analyses

A placebo blank of the composition [9]: 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 made and its solution was prepared as described 'procedure for capsules', and then subjected to analysis.

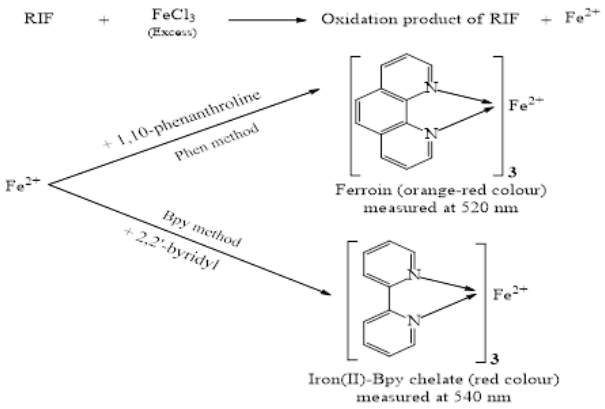
To assess the role of the inactive ingredients on the assay of RIF, a synthetic mixture was separately prepared by adding 10 mg of RIF to the 10 mg placebo mentioned above. The drug was extracted and solution was prepared as described under the 'procedure for capsules'. The solutions after appropriate dilution were analyzed following the recommended assay procedures.

Procedure for analysis of spiked human urine

The method of Salem et al. [12] was used to prepare spiked urine sample. Ten mg of the pure RIF and 10 mL of urine sample were transferred into a separating funnel, mixed well till 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 to dryness. The resulting residue was reconstituted with water and diluted to 100 mL with water. The resulting urine solution (100 µg mL^{-1} in RIF) was diluted to 15, 25 & 35 µg mL^{-1} in both methods with water and proceeded further as above.

4. Results

Iron(III) salts are very useful in the spectrophotometric assay of many pharmaceuticals [41-47]. In the present study, the reaction proceeds through the reduction of iron(III) to iron(II) by RIF and the subsequent formation of the intense orange-red colored chelate with either *o*-phenanthroline or 2,2'-bipyridyl as shown in scheme 1. The absorption spectra of the coloredspecies showed characteristic λ_{max} values as shown in Fig. 2. The increasing absorbance values at 520 nm in phen method and at 540 nm in bipy method were plotted against the concentration of RIF to obtain the calibration plots.



Scheme 1. Possible reaction scheme for the proposed methods

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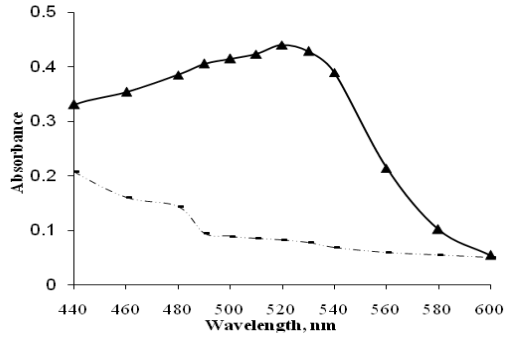

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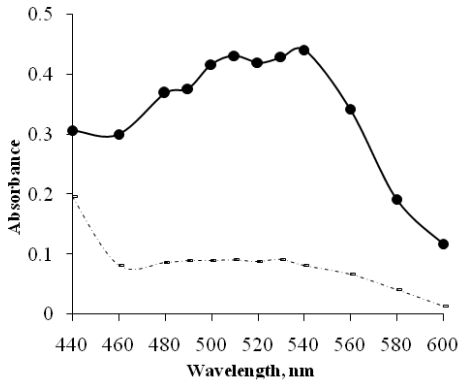


(b)

Figure 2.Absorption spectra; (a) RIF-phen complex (-▲-) (20 µg mL^{-1} RIF), (b) RIF-bipy complex (-●-) (30 µg mL^{-1} RIF) and blanks (- - -)

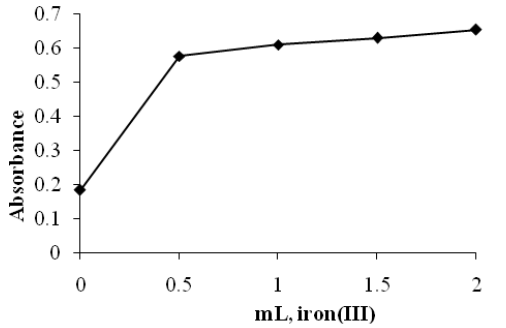
5. Discussion

Method development

The experimental conditions were established by varying each parameter individually and observing its effect on the absorbance of colored species.

Effect of reagent concentration

The effect of reagent volume was studied in the range from 0.5-3.0 mL, and it was found that 1.0 mL of 0.01M Fe^{3+} was sufficient for both methods and 2.0 mL of 0.01Mchelatingagentssolutions were necessary to obtain the maximum and reproducible absorbance for 30 µg mL^{-1} RIF in phen method and 20 µg mL^{-1} of RIF in bipy method as shown in Figure 3 & 4. Smaller amounts resulted in incomplete complex formation; increased concentration of reagents gave constant absorbances. One mL of 0.02M phosphoric acid was found necessary to give the required pH and stabilize the absorbance value.



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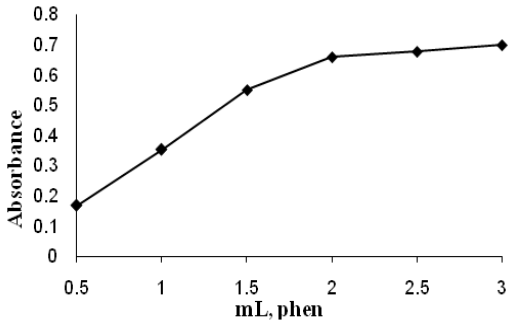
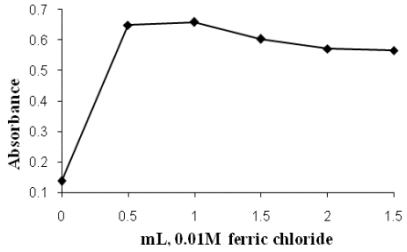


(b)

Figure 3.Effect of: (a) iron(III) and (b) 1,10-phenanthroline (20 µg mL^{-1} RIF) in phen method



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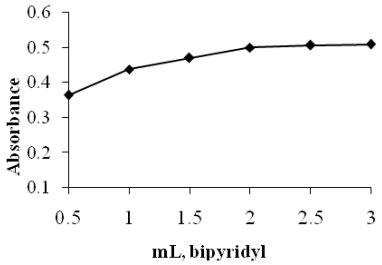

(b)

Figure 4.Effect of: (a) iron(III) and (b)bipyridyl (30 µg mL^{-1} RIF) in bipy method

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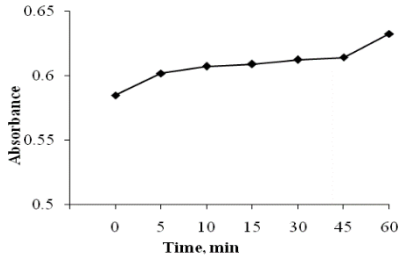
Reaction time and stability of complexes

As shown in Figure 5, the reaction time required for full color development was 10 min in phen method & 5 min in bipy method and the color was stable for at least 45 minutes in phen method and up to one day in bipy method.



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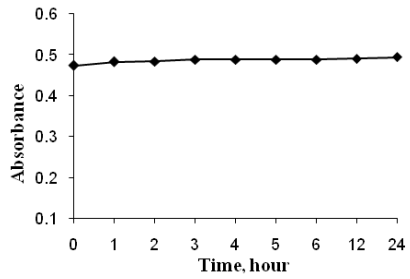


(b)

Figure 5.Reaction time and stability: (a)Phenmethod (20 µg mL^{-1} RIF), (b)Bipy method (30 µg mL^{-1} RIF)

Method validation

Linearity, sensitivity, limits of detection and quantification

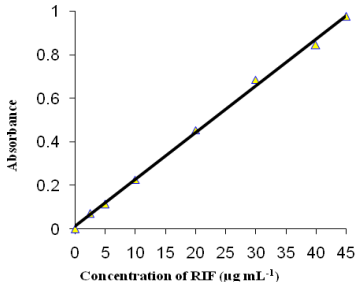
A linear correlation was found between absorbance at λ_{max} and concentration of RIF in the ranges given in Table 1 (Fig. 6). The graphs are described by the regression equation:

$$Y = a + bX$$

(where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in µg mL^{-1}). Regression analysis of the Beer's law data using the method of 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 of both methods are also given in Table 1. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [48] using the formulae:

LOD = 3.3 S/b and LOQ = 10 S/b,

(where S is the standard deviation of blank absorbance values, and b is the slope of the calibration plot) are also presented in Table 1. The high values of molar absorptivity (ε) and low values of Sandell sensitivity and LOD indicate the high sensitivity of the proposed methods.



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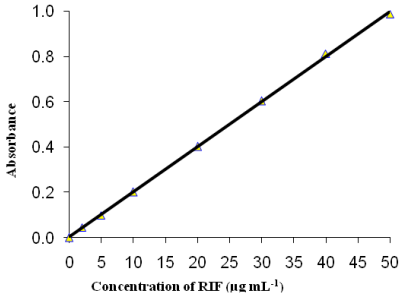

(b)

Figure 6.Calibration plot: (a) Phen method and (b) Bipy method

Table 1:Sensitivity and regression parameters

Parameter	Phenmethod	Bipy method
λ_{max}, nm	520	540
Reaction time, min	10	5
Color stability	45 min	≥ 24 hr
Linear range, µg mL^{-1}	2.5–45	2.5-50
Molar absorptivity (ε), L mol^{-1} cm^{-1}	1.91×10^{4}	1.68×10^{4}
Sandell sensitivity*, µg cm^{-2}	0.0433	0.0491
Limit of detection (LOD), µg mL^{-1}	0.30	0.36
Limit of quantification (LOQ), µg mL^{-1}	0.92	1.09
Regression equation, Y**		
Intercept (a)	0.0177	0.0038
Slope (b)	0.0213	0.0199
Standard deviation of a (S_a)	9.98×10^{-2}	9.98×10^{-2}
Standard deviation of b (S_b)	2.40×10^{-3}	2.23×10^{-3}
Regression coefficient (r)	0.9989	0.9998

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*Limit of determination as the weight in µg mL^{-1} 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, X is concentration in µg mL^{-1}, a is intercept and b is slope

Precision and accuracy

The assays described under "general procedures" were repeated seven times within the day to determine the repeatability (between-day precision) and five times on different days to determine the intermediate precision (day-day precision) of the methods. These assays were performed for three levels of analyte. The results of this study are summarized in Table 2. The percentage relative standard deviation (%RSD) values were ≤ 1.31% (between-day) and ≤ 1.94% (day-day) indicating high precision of the methods. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and taken concentrations for PYL. Bias {bias% = [(Concentration found - known concentration) × 100/known concentration]} was calculated at each concentration and these results are also presented in Table 2. Percent relative error (%RE) values of ≤ 2.36% demonstrate the high accuracy of the proposed methods.

Table 2:Evaluation of between-day and day-day accuracy and precision

Method	RIF taken (µg mL^{-1})	Between-day accuracy and precision (n=7)			Day-day accuracy and precision (n=7)		
		RIF found[a] (µg mL^{-1})	RSD[b] %	RE[c] %	RIF found (µg mL^{-1})	RSD[b] %	RE[c] %
Phen	15	14.74	1.06	1.74	15.24	1.21	1.60
	25	24.41	0.99	2.36	25.21	1.29	0.84
	35	35.37	1.24	1.06	35.35	1.92	1.00
Bipy	15	14.80	1.31	1.34	15.30	1.19	2.00
	25	25.30	1.25	1.20	24.52	1.33	1.92
	35	34.69	1.01	0.89	35.41	1.94	1.17

[a]Mean value of 7 determinations; [b] Relative standard deviation (%); [c] 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 percent recoveries of 98.5±1.63 and 101.9±1.02 (n=5) for phen method and bipy method, respectively, demonstrated the accuracy as well as the precision of the proposed methods and complement the findings of the placebo blank analysis with respect to selectivity.

Robustness and ruggedness

The robustness of the methods was evaluated by making small incremental changes in the volume of iron(III) solution & reaction times and the effect of the changes was studied on the absorbance of the colored systems. The changes had negligible influence on the results as revealed (in Table 3) by small intermediate precision values expressed as %RSD (≤ 1.94%). Methods' ruggedness was demonstrated having the analysis done 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 in the range 0.78-2.13% indicating acceptable ruggedness. The results are presented in Table 3.

Table 3:Method robustness and ruggedness expressed as intermediate precision (%RSD)

Method	Nominal concentration	Robustness		Ruggedness	
		Parameters altered		Inter-analysts (*n=3*)	Inter-instruments (*n=3*)
		Reaction time* (*n=3*)	Iron(III) volume[#] (*n=3*)		
Phen	15	1.94	1.12	0.78	2.13
	25	0.75	1.74	1.51	1.72
	35	1.24	0.95	1.25	2.05
Bipy	15	1.34	1.52	1.58	1.02
	25	1.69	1.14	1.85	1.07
	35	0.95	1.25	1.12	0.95

[#] Volumes of iron(III) solution were 1±0.1 mL in both methods.

*Reaction times were: 10±1 min in phen method & 5±1 min in bipy method.

Application to capsules

The proposed methods were applied to the quantification of RIF in commercial capsules. The results were compared with those obtained by the officialEuropean Pharmacopoeia method [49]. According to which capsule extract equivalent to 100 µg mL^{-1} RIF was prepared in methanol and 5 mL of this extract was diluted to 10 mL with phosphate buffer of pH 7.4,

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and absorbance measured at 475 nm *vs* buffer. Statistical analysis of the results did not detect any significant difference between the performance of the proposed methods and reference method with respect to accuracy and precision as revealed by the Student's *t*-value and variance ratio *F*-value. The results of assay are given in Table 4.

Table: 4.Results of analysis of formulations by the proposed methods

Formulation analyzed	Label claim (mg/ capsule)	Reference method	Found* (%label claim±SD)	
			Proposed methods	
			Phen method	Bipy method
R-Cin 300	300	99.5±1.07	99.1±1.25 *t* = 0.55 *F* = 1.36	100.6±1.63 *t* = 1.26 *F* = 2.32
R-Cin 450	450	101.3±0.95	100.8±1.59 *t* = 0.60 *F* = 2.80	102.5±1.27 *t* = 1.69 *F* = 1.79

*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.

Recovery study

To further assess the accuracy of the methods, recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure RIF at three different levels (50, 100 and 150%) of the content present in the capsule powder (taken) and the total was found by the proposed methods. Each test was repeated three times. In all the cases, the recovery percentage values ranged between 99.1 and 102.4% with standard deviation in the range 0.59-1.51%. 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

Method	Formulation studied	RIF in capsule µg mL^{-1}	Pure RIF added µg mL^{-1}	Total found µg mL^{-1}	Pure RIF recovered * Percent±SD
Phen	R-Cin 300	11.89	6	18.16	101.5±1.20
		11.89	12	23.79	99.6±0.94
		11.89	18	30.52	102.1±0.59
	R-Cin 450	12.1	6	18.53	102.4±1.14
		12.1	12	23.93	99.3±1.51
		12.1	18	30.52	101.4±1.24
Bipy	R-cin 300	12.07	6	17.98	99.5±1.25
		12.07	12	24.19	100.5±1.09
		12.07	18	30.43	101.2±0.71
	R-Cin 450	12.3	6	18.43	100.7±1.05
		12.3	12	24.62	101.3±1.34
		12.3	18	30.03	99.1±1.05

*Mean value of three determinations.

Application to spiked human urine

The proposed methods were further extended to the assay of RIF in spiked human urine samples. The results of the study are summarized in Table 6 and are satisfactorily accurate and precise in the range 101.8-103.1% with standard deviation 0.57-1.12%.

Table 6:RIF determination in spiked urine sample, n = 5

Method	Spiked concentration (µg mL^{-1})	Concentration found* (µg mL^{-1})	%Recovery ±SD*
Phen	15	15.43	102.9±0.85
	25	25.45	101.8±0.57
	35	36.08	103.1±0.74
Bipy	15	15.34	102.3±1.07
	25	25.78	103.1±0.63
	35	35.63	101.8±1.12

*Mean value of five determinations of RIF.

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References

1. Hilman, A.G., Rall, T., Neir, A., Taylor, P., 1996. The Pharmaceutical Basis of Therapeutics, New York, McGraw Hill.

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www.jchr.org

JCHR (2024) 14(1), 1945-1954 | ISSN:2251-6727

2. Maher, H.M., Youssef, R.M., 2008. *Chemometr. Intell. Lab. Syst.* 94, 95.
3. Alonso, M.A.L.O., Dominguez Renedo, M.J., Arcos, M., 2001. *Anal. Chim. Acta.* 449, 167.
4. Sastry, C.S.P., Divakar, T.E., Prasad, U.V., 1986. Spectrophotometric determination of rifampicin with uranyl or thorium(IV).*J. Inst. Chem. (India)* 58, 17-18.
5. Gandhi, T.P., Patel, A.A., Patel, P.R., Patel, V.C., 1978. Colorimetric estimation of rifampicins in formulations and biological fluids by metallic ions. *Indian Drugs* 16, 10-12.
6. Sadeghi, S., Karimi, E., 2006. Spectrophotometric determination of rifampicin through chelate formation and charge transfer complexation in pharmaceutical preparation and biological fluids. *Chem. Pharm. Bull.* 54, 1107-1112.
7. Sastry, C.S.P., Divakar, T.E., Prasad, U.V., 1985.Spectrophotometric determination of rifampicin with some metal ions. *Indian drugs* 22, 604-606.
8. Reddy, B.S., Sastry, C.S.P., 1983. Ion-pair extraction method for ethambutol, ethionamide and rifampicin determination. *J. Inst. Chem. (India),* 55, 69-70.
9. Barsoum, N.B, Kamel, M.S., Diab, M.M.A., 2008.Spectrophotometric determination of isoniazid and rifampicin from pharmaceutical preparations and biological fluids.*Res. J. Agri. Bio. Sci.* 4, 471-484.
10. Finkel, J.M., Pittillo, R.F., Mellett, L.B., 1971. Fluorometric and microbiological assays for rifampicin and the determination of serum levels in the dog.*Chemoth.* 16, 380–388.
11. Hammam, E., Beltagi, A.M., Ghoneim, M.M., 2004. Voltammetric assay of rifampicin and isoniazid drugs, separately and combined in bulk, pharmaceutical formulations and human serum at a carbon paste electrode. *Microchem. J.* 77, 53–62.
12. Salem, A.A., Mossab, H.A., Barsoum, B.N., 2005. Quantitative determinations of levofloxacin and rifampicin in pharmaceutical and urine samples using nuclear magnetic resonance spectroscopy.*Spectrochim. Acta. Part A*, 62, 466–472.
13. Khuhawar, M.Y., Rind, F.M.A., 2002. Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood.*J. Chromatogr. B*, 766, 357–363.
14. Walubo, A., Smith, P., Folb, P.I., 1994. Liquid chromatographic determination of isoniazid, pyrazinamide and rifampicin from pharmaceutical preparations and blood.*J. Chromatogr. B*, 658, 391-396.
15. He´ctor, G.C., Alejandro, O.C., 1999. Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration.*J. Pharm. Biomed. Anal.* 20, 681–686.
16. Espinosa, M.A., Acedo, V.M.I., Muñoz, D.P.A., Salinas, F., Cañada, C.F., 2001. Comparative study of partial least squares and a modification of hybrid linear analysis calibration in the simultaneous spectrophotometric determination of rifampicin, pyrazinamide and isoniazid.*Anal. Chim. Acta*. 427, 129–136.
17. Marcellos, L.F., Faria, A.F., Souza, M.V.N., Almeida, M.R., Sabin, G.P., Poppi, R.J., Oliveira, M.A.L., 2012. Simultaneous analysis of first-line anti-tuberculosis drugs in tablets by UV spectrophotometry compared to capillary zone electrophoresis.*Cent. Eur. J. Chem.* 10, 1808-1816.
18. Kakde, R.B., Kasture, A.V., Wadodkar, S.G., 2002. Spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations.*Ind. J. Pharm. Sci*. 64, 24-27.
19. Benetton, S.A., Kedor-Hackmann, E.R.M., Santoro, M.I.R.M., Borges, V.M., 1998. Visible spectrophotometric and first-derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations.*Talanta*, 47, 639–643.
20. Rote, A.R., Sharma, A.K., 1997. Simultaneous spectrophotometric determination of rifampicin, isoniazid and pyrazinamide by first-derivative UV spectrophotometry in combined pharmaceutical dosage forms.*Ind. J. Pharm. Sci*. 59, 119-123.
21. Rasha, M.Y., Hadir,M.M., 2008. A new hybrid double divisor ratio spectra method for the analysis of ternary mixtures. *Spectrochim. Acta. Part A*, 70, 1152–1166.
22. Wahdan, T., 2005.Voltammetric method for the simultaneous determination of rifampicin and isoniazid in pharmaceutical formulations.*Chem. Anal. (Warsaw)*, 50, 457-464.
23. Karim, A.Z., Payam, S., 2010. Simultaneous polarographic determination of isoniazid and rifampicin by differential pulse polarography method and support vector regression.*Anal. Chim. Acta.* 55, 6570–6576.
24. Alonso, M.A.L., Dom´ınguez, O.R., Arcos, M.J.M., 2001. Resolution of ternary mixtures of rifampicin, isoniazid and pyrazinamide by differential pulse polarography and partial least squares method. *Anal. Chim. Acta.* 449, 167–177.
25. Alonso, M.A.A., Kauffmann, J.M., Arcos, M.J.M., 2003. HRP-based biosensor for monitoring rifampicin. *Biosens. Bioele.* 18, 1165-1171.
26. Baoxin, L., Yuezhen, H., Jiagen, L., Zhujun, Z., 2005. Simultaneous determination of rifampicin and isoniazid by continuous-flow chemiluminescence with artificial neural network calibration.*Anal. Bioanal. Chem.* 383, 817–824.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 1945-1954 | ISSN:2251-6727

27. Yong, M., Bo-Tao, Z., Li-Xia, Z., Guang-Sheng, G., Jin-Ming, L., 2008. Determination of rifampicin by peroxomonosulfate-cobalt(II) chemiluminescencesystem. *Chin. J. Chem.* 26, 905-910.
28. Calleri, E., De Lorenzi, E., Furlanetto, S., Massolini, G., Caccialanza, G., 2002. Validation of a RP-LC method for the simultaneous determination of isoniazid, pyrazinamide and rifampicin in a pharmaceutical formulation. *J. Pharm. Biomed. Anal*. 29, 1089–1096.
29. Gunasekaran, S., Sailatha, E., 2009.Estimation of pyrazinamide, isoniazid and rifampicinin pharmaceutical formulations by high performance liquid chromatography method.*J. Chem.* 21, 3561-3566.
30. Shewiyoa, D.H.,Kaaleb, E., Rishab, P.G., Dejaegherc, B., Smeyers, V.J., Vander, H.Y., 2012. *J. Chromatogr. A*, 1260, 232–238.
31. Ali, J., Ali, N., Sultana, Y., Baboota, S., Faiyaz, S.,2007. Development and validation of a stability-indicating HPTLC method for analysis of antitubercular drugs.*Acta. Chromatogr*. 18, 168-179.
32. Shah, Y., Khanna, S., Jindal, K.C.,Dighe, V.S., 1992. Determination of rifampicin and isoniazid in pharmaceutical formulations by HPLC.*Drug. Dev. Ind. Pharm*. 18, 1589-1596.
33. Younghee, H., Sunmi, S., 2001.Electrochemical behaviour and differential pulse polarographic determination of rifampicin in the pharmaceutical preparations.*Arch. Pharm. Res.* 24, 100-104.
34. Lomillo, M.A.A., Renedo, O.D., Martinez, M.J.A., 2002. Optimization procedure, applying the experimental-design methodology, for the determination of rifampicin after metal complexation by differential pulse adsorptive stripping voltammetry.*Helvet. Chim. Acta*. 85, 2430-2439.
35. Shereen, M.G., Salah, M.B., Abdel-Hamid, M.E., 1992. Comparative spectrophotometrc analysis of rifampicin by chelate formation and charge-transfer complexation. *Anal. Lett.* 25, 725-743.
36. Sastry, C.S.P., Divakar, T.E., Prasad, U.V., 1985. Spectrophotometric determination of rifampicinwith chloranil. *Ind. J. Pharm. Sci.* 47, 45-46.
37. Divakar, T.E., Sunitha, S., Deepthi, G.K., Benzamin, T., Babu, N.P., 2012.*Int. J. Chem. Environ. Pharm. Res.* 3, 64-67.
38. Shukla, I.C., Dwivedi, P.K., Kumar, S., Singh, B.K., Dubey, A., 2007.Application of ammonium metavanadate for the determination of some tubercular and adrenocortical steroid drugs. *J. Ind. Chem. Soc.* 84, 100-102.
39. Rao, G.R., Murty, S.S.N., Rao, E.V., 1985. Spectrophotometric determination of rifampicin in pharmaceutical dosage forms. *Indian Drugs*, 22, 484-488.
40. Divakar, T.E., Prasad, U.V., Sastry, C.S.P., 1985.Spectrophotometric estimation of tetracyclines and rifampicin using p-N,N-dimethylphenylenediamine and chloramine T. *Indian Drugs*, 22, 328-329.
41. Zenita, D.O., Basavaiah, K., 2010. Validated spectrophotometric determination of pantoprazole sodium in pharmaceuticals using ferric chloride and two chelating agents. *Chemtech*, 2, 624-632.
42. Vinay, K.B., Revanasiddappa, H.D., Zenita, D.O., Basavaiah, K., 2010. Spectrophotometric determination of etamsylate in pharmaceuticals using ferric chloride based on complex formation reactions. *Chem. Ind. Chem. Engg. Quart.* 16, 1−9.
43. Ramesh, P.J., Basavaiah, K., Rajendraprasad, N., Zenita, D.O., Vinay, K.B., 2012. Spectrophotometric determination of ofloxacin in pharmaceuticals by redox reaction. *J. Appl. Spectr*. 78, 383-391.
44. Murthy, V.R., Acharyulu, M.L.N., Srinivas, B.V., Reddy, T.S., Srama, G.V.S.R., 2013. *Eur. J. Appl. Engg. Sci. Res.* 2, 9-13.
45. Al-Sabha, T.N., 2007. *J. Edu. Sci.* 19, 25-35.
46. Gowda, A.A., Hassan, W.S., 2008. Spectrophotometric determination of etodolac in pure form and pharmaceutical formulations. *Chem. Cent. J.* 2, 1-8.
47. Imam, P.S., Fazlu, R., Taqui, M., Sanjyothi, Y., Shravan, K.A., 2012. *Int. J. Pharm & Ind. Res.* 2, 47-50.
48. 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 dated 06 November 1996, incorporated in november 2005, London.
49. Europen Pharmacopoea Monographs, 8.0, 3165-3166.

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Table 7: Performance characteristics of the published visible spectrophotometric methods.

Sl. No	Reagent/reaction	λ_{max} (nm)	Linear range (μg mL^{-1}) ε ($Lmol^{-1}cm^{-1}$)	Remarks	Ref.
1	Complex formation with a) Uranyl acetate b) Thorium nitrate	 490 525	-	-	4
2	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	5
3	C-T complex formation with DDQ TCNQ *p*-chloranil	 584 680 560	 5-140 5-120 15-200	Less sensitive, organic solvent medium required	6
	Complex formation with iron(III)	540	10-240	Less sensitive, requires extraction step and use of organic solvent	
4	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	7
5	Ion-pair formation with a) alizarin violet 3B b) alizarin brilliant violet R	 560	 -	Requires critical pH adjustment, extraction step and use of organic solvent	8
6	Redox complexation reaction using NBS-KI-starch	572	0.5-15.5	-	9
7	a) Chelate formation with Cu^{2+} b) C-T complex formation with halogenated quinones	-	40-100	Less sensitive, narrow linear range and use of organic solvent	35
8	C-T complex formation with *p*-chloranil	500	5-50	Indicial adjustment of pH required	36
9	C-T complexation with chloranilic acid		-	uses organic solvent medium	37
10	Redox reaction with ammonium metavanadate	-	-	-	38
11	Redox reaction with a) DCQC b) $K_2Cr_2O_7$	 545 540	 0-110 0-210	Less sensitive	39
12	Oxidative condensation reaction with PDPD and Chloramine-T	640 520	-	Requires extraction with organic solvent	40
13	**a) Phen method** **b) Bipymethod**	**520** **540**	**2.5-45** **1.91×10^4** **2.5-50** **1.68×10^4**	**Sensitive, wide linear dynamic range, use of eco-friendly reagents, uses aqueous solution**	**Present work**

*NBS-N-bromosuccinide, DDQ-di-chloro,di-cyano-*p*-benzoquinone, TCNQ-tetracyanoquinone.