Research Journal of Agriculture and Biological Sciences, 4(5): 471-484, 2008

Spectrophotometric Determination of Isoniazid and Rifampicin from Pharmaceutical Preparations and Biological Fluids

[1]Barsoum.N.Barsoum, [2]Manal S. Kamel and [1]Mohamed M.A. Diab

[1]Department of chemistry, Faculty of Science, Cairo University, Egypt
[2]Department of applied organic chemistry, Microanal. Lab.,
National Research Centre, Cairo, Egypt.

Abstract: Two methods are described for the determination of the commonly used antimycobacterial drugs such as Isoniazid and Rifampicin in their pure forms, pharmaceutical preparations and biological fluids. Method A can be used as direct UV spectrophotometric measurement and the absorbencies were measured at wavelengths 264 and 474 nm for Isoniazid and Rifampicin respectively. Method B based on the reaction of drugs with N-bromosuccinimide (NBS), then the excess of NBS was reacted with KI, the liberating iodine is determined at wave length 572 nm. The methods are rapid, simple and do not require any separation step. The recovery average was 99.86% for Isoniazid and 99.02% for Rifampicin. Regression analysis of Beer's law plots showed good correlation in the concentration ranges between (2-42 and 0.82-65.38μg ml^{-1}) in method A and from (0.1 – 3.4 and 0.5-15.5 μg ml^{-1}) in method B, respectively. The results were in good agreement with those obtained by the official and reported methods.

Key words: Spectrophotometric; Isoniazid; Rifampicin; *N*-bromosuccinimide (NBS); Pharmaceutical preparations; Biological fluids.

INTRODUCTION

Isoniazid is Isonicotinic acid hydrazide[1] used and Rifampicin as antimyco- bacterial drugs which attracted special attention for their therapeutic importance[2] in the treatment of T.B.[3]. Several methods have been reported for the determination of Isoniazid includes HPLC[4,5], GC[6,7], Chemiluminescence[8,9], Electrometry[10,11], Titrimetry[12,13] and Spectrophotometry[14,16].

Several methods also have been reported for the determination of Rifampicin includes HPLC[17,18], Chemiluminescence[19,20], Electrometry[21,22], and Spectrophotometry[23,25]. The published methods for the determination of each drug is relatively little. The present study was under taken to show the light on the determination of the drugs (Fig. 1). This paper describes simple and sensitive spectrophotometric methods for the evaluation of Isoniazid and Rifampicin in pure, dosage forms and biological fluids. It expected that the developed method would be simpler, sensitive, low costing, low reagent consumption and achieve higher accuracy and precision compared to previously published methods.

MATERIALS AND METHODS

Apparatus: Perkin elmer Lambada 20 double beam self recording spectro photometer with 10 mm quartz cell, connected to PC software.

HANA pH meter HI 8417 with pH sensitivity of ± 0.05 pH units.

Perkin elmer HPLC has μBondapak C_{18} (300x3.9 mm) column. The flow rates are about 1.5 ml per minutes equipped with 254 nm detectors for Isoniazid determinations.

Perkin elmer HPLC has μl-Tracarb 7OSD (250x4.6mm). The flow rates are about 1.5 ml per minutes equipped with 254 for Rifampicin.

Materials: N-bromosuccinamide (NBS), Triethanolamine and Hydrazine dichloride, purchased from Merk – shuchardt, England. Potassium iodide (KI) purchased from Fein chemie, Germany. Starch soluble, Urea and pyridoxine (vitamin B_6) purchased from BDH laboratories, Poole, England. Iodine resublimed. EDTA purchased from Win lab. U.K. Sodium oxalate purchased from Mallinckrodt chemicals. U.S.A. Sodium Chloride purchased from Fluka company, Germany. Acetonitrile and Methanol HPLC grade purchased from

Corresponding Author: Manal S. Kamel, Department of applied organic chemistry, Microanal. Lab., National Research Centre, Cairo, Egypt.
E-mail: manalskamel@yahoo.com

Scharlau chemie, Spain. Authentic samples of Isonicotinic hydrazide (Isoniazid) powder supplied from Fluka company, Germany. Rifampicin. Supplied from El-Nasr pharmaceutical company, Egypt. Market samples of Isocid 50 mg tablets, Isocid fort 200 mg tablets produced by CID Company Egypt. Rimactane 300 mg capsules produced by Novartis pharma., Egypt. Riozid (300 mg Rifampicin + 150 mg Isoniazid) Capsules, Medical union pharma ceuticals (MUP), Abu-sultan, Ismailia, Egypt. Rimactazid, (300 mg Rifampicin + 150 mg Isoniazid) Capsules, Novartis Parma, Egypt. All supplied from local market.

Biological fluids, Random healthy men urine samples freshly collected. Random Plasma samples collected from human blood samples using sodium oxalate as anti coagulant. All were supplied from medical laboratories where all patients possess tuberculin negative intradermal test subsequently not treated with any antimycobacterial drugs.

All solutions were freshly prepared. Double distilled water was used. Standard Isoniazid solutions with different concentrations $1x10^{-4}$M, $5x10^{-4}$M and $1x10^{-3}$ M. Standard $1x10^{-4}$M and $1x10^{-3}$ M Rifampicin aqueous solution. 2 x 10^{-4}M NBS aqueous solution. 2% KI aqueous solution. 1% Starch solution prepared by mixing 1 gm of soluble starch with 5 ml distilled water then completed to 100 ml boiled distilled water, till clear solution obtained and left to be cold before used. Series of phosphate buffers pH 2.5 – 10. Saturated Sodium chloride solution.

Procedures for Method A:

General Procedure: Transfer 1–6 ml (1 x 10^{-3} M) from standard solution of Isoniazid or Rifampicin in to 10 ml measuring flask and completed by distilled water. Absorbencies were measured at wave lengths 264 and 474 nm for Isoniazid and Rifampicin respectively against distilled water blank (Figure 3). Each measurement was repeated for five times and compared statistically with the official methods[2].

For Pharmaceutical Preparations: Twenty tablets were weighted and ground to finely divided powder.or the powder from ten capsules were obtained. An accurate weight of the powder contain 6.8 and 41.14 mg of Isoniazid or Rifampicin was dissolved in to 50 ml distilled water to produce 1 x 10^{-3} M final concentration. The solution was then filtered off, then general procedure were followed.

For Isoniazid in Biological Fluids: 5 ml of biological fluid (urine or plasma) was spiked with 0.05 - 2 mg of Isoniazid powder. 1 ml biological fluid was mixed with 1 ml saturated Sodium chloride solution then diluted to 10 ml in 10 ml measuring flask. The resulting solution was vortexing for 1 minute and centrifuged for 5 minutes at 3000 rpm in ambient temperature. 1.0 ml of the supernatant was transferred in to 10 ml volumetric flask. 1–6 ml of $1x10^{-3}$M Isoniazid aqueous solution was transferred in to 10 ml calibrated flasks. The absorbencies were measured at wave length 264 nm. The results obtained multiplied by dilution factor 100 gives the real concentration of the drug in the biological fluid. Each measurement was repeated for five times and compared statistically with the official HPLC method[2].

For Rifampicin in Biological Fluids: 10 ml of urine sample was spiked with 0.082 – 0.66 mg of Rifampicin powder. Centrifuged at 150 rpm for 5 minutes then the supernatant measured directly at 474nm. The general procedure was followed. 10 ml of plasma sample was spiked with 0.822 – 6.6 mg of Rifampicin powder. 1.0 ml of the resulting solution was transferred in to 10 ml volumetric flask .Centrifuged at 150 rpm for 5 minutes then the general procedure was followed. The results obtained from plasma fluid multiplied by dilution factor 10 gives the real concentration of the drug in the plasma fluid. Each measurement was repeated for five times and compared statistically with the official HPLC method[2].

Procedures for Method B:

Stoichiometry of Reaction Between N-bromosuccinimide (NBS) and Isoniazid or Rifampicin: Stoichiometry of the drugs with NBS was determined by applying the molar ratio method described by Yoe and Jones[26], (Figure 2). The concentration of the reagent is kept constant at ($2.5x10^{-4}$M) per 10 ml while the drug concentration is regularly varied. Successive aliquots 0.4-2.4 ml of the standard $1x10^{-4}$M Isoniazid or Rifampicin solutions was transferred in to 10 ml measuring flasks. Each measuring flask contains 2.5 ml of 2x 10^{-4} M NBS solution then left for 30 minutes. 1ml of (2%) KI solution was added then left for ten minutes. 1ml of the Starch solution was added. The absorbencies of the resulting solutions were measured against distilled water as reagent blank at wave length 572 nm.

General Procedure: Transfer 1–6 ml of $1x10^{-4}$ M from standard solution of Isoniazid or Rifampicin in to 25 ml measuring flasks. In each measuring flask 6.5 ml of NBS solution was added (after 30 minutes), 2 ml KI solution was added then left for 10 minutes. 2 ml starch solution was added and completed by distilled water. The absorbencies were measured at wave length 572 nm against distilled water reagent blank (Figure 4). Each measurement was repeated for five times and compared statistically with the official HPLC method[2].

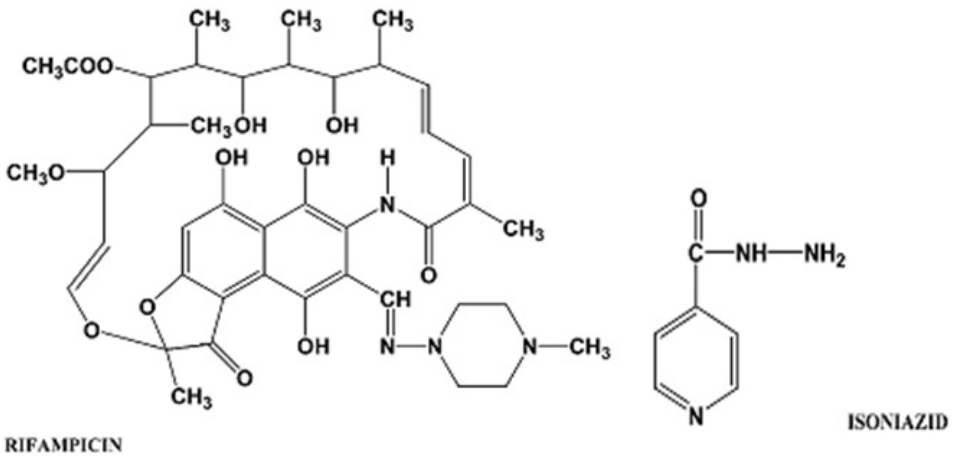


Fig. 1: Structure of the studied drugs.

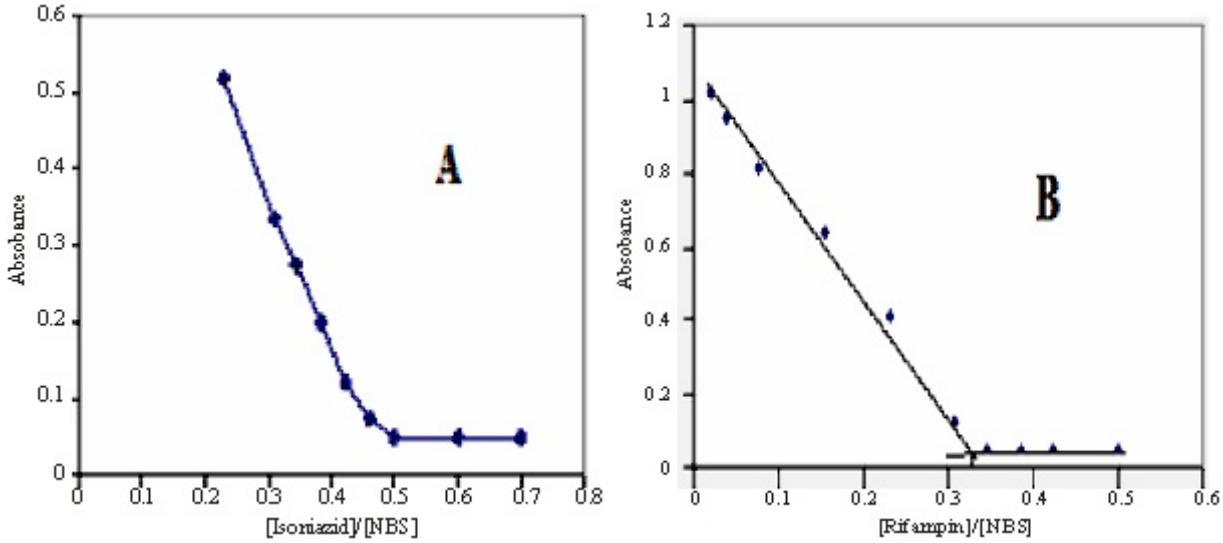


Fig. 2: Molar ratio plotting of A) Isoniazid and B) Rifampicin drugs with NBS.

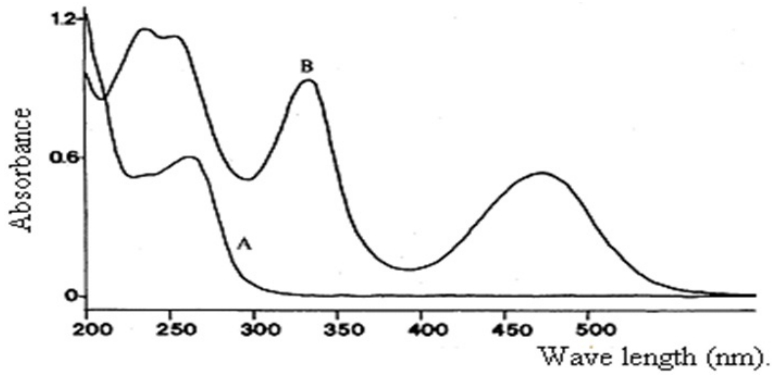


Fig. 3: Absorption spectra of (A) Isoniazid 20µg ml^{-1} and (B) Rifampicin 30 µg ml^{-1} in phosphate buffer solution pH 7.4

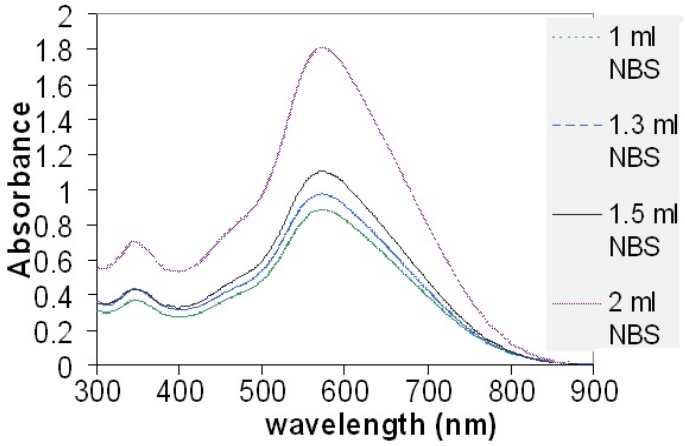


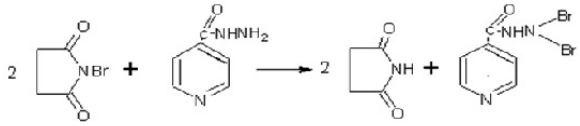
Fig. 4: Absorption spectra of different volumes of NBS solution.

Procedure for Pharmaceutical Preparations: Twenty tablets were weighted and ground to finely divided powder or the powder from ten capsules were obtained. An accurate weight of the powder contain 0.68 and 4.114 mg of Isoniazid or Rifampicin was dissolved in to 50 ml distilled water to produce 1 x 10^{-4} M final concentration. The solution was then filtered off, then the general procedure was followed.

Procedure for Biological Fluids: 5 ml of biological fluid (urine or plasma) was spiked with 0.5-7.0 mg of Isoniazid or 1.0 -60 mg of Rifampicin powder. 1 ml biological fluid was mixed with 1 ml saturated Sodium Chloride solution then diluted to 10 ml in 10 ml measuring flask. The resulting solution was vortexing for 1 minute and centrifuged for 5 minutes at 3000 rpm in ambient temperature. 1 ml of the supernatant was diluted to10 ml in 10 ml measuring flask. 2.5 ml of the resulting solution was transferred in to 25 ml volumetric flask in each calibrated flask 6.5 ml of NBS solution was added then left for 30 – 60 minutes, 2 ml KI solution was added then left for 10 minutes. 2 ml of starch solution was added, absorbencies were measured at wave length 572 nm against distilled water reagent blank. The results obtained multiplied by dilution factor 1000 gives the real concentration of the drug in the biological fluid. Each measurement was repeated for five times and compared statistically with the official HPLC method.

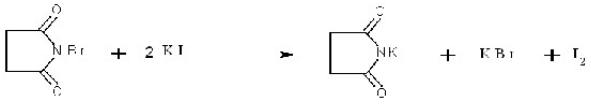
RESULTS AND DISCUSSION

The reaction between Isoniazid and NBS had applied by Barakat and Shaker[27] in the titrimetric determination of Isoniazid in aqueous acid medium with methyl red indicator. They state that the reaction was quantitative in accordance with Equation 1. Which coincident with the molar ratio plot shown in (Figure 2, A) which indicates that the stiochiometric ratio NBS: Isoniazid is (2:1).



Equation 1: Proposed reaction pathway between Isoniazid and NBS.

There are three hydroxyl groups in the Rifampicin structure may be oxidized and the liberated bromides may brominate the nitrogen atom adjacent to naphthaliene skeleton replace its hydrogen atom. Also brominate the nitrogen atom adjacent to the piperazine ring and the attached olefinic bond to this atom in the structure. Which may explain the stochiometric ratio (3:1) between NBS and Rifampicin as shown in (Fig. 2B). Then the excess amount of NBS was reacting with Potassium Iodide solution liberating Iodine which gives a blue color with starch solution according to the Equation 2.



Equation 2: Proposed reaction pathway between NBS and KI.

Determination of the Suitable Conditions for the Methods: The different experimental parameters affecting the color development were extensively studied to determine the optimal conditions for this procedure as flow.

Selection of Wavelength: In method A, Isoniazid and Rifampicin exhibit characteristic peaks at wave lengths 264 and 474 respectively (Fig. 3). The absorbance of these peaks has been used to determine the concentration of each drug in its pure form, commercial pharmaceutical preparations and some body fluids. In method B, The proper wavelength for the resulted Iodine –Starch complex species in the proposed pathway was selected according to the absorption spectra shown in (Fig. 4) which indicates that the resulting Iodine – Starch complex species gives maximum absorbance at wave length (λ) 572 nm.

Optimum NBS Volume: The optimum volume of Starch which was sufficient to give a reasonable stable maximum absorbance with the Iodine liberated from 6.5 ml 2 x 10^{-4} mol L^{-1} NBS aqueous solution in 25 ml calibrated flask corresponds to 5.2 x 10^{-5} M NBS as final concentration found to be above 2 ml 1% starch solution as shown in (Fig. 4).

Effect of pH: In method A, The absorbance of the peak obtained from 27.24 and 24.68 μg ml^{-1} of Isoniazid and Rifampicin respectively in different Phosphate buffer media that stayed in different time periods. (Fig. 5) depicts that pH ranges 6.0-7.5 after 1 day was the optimum for Isoniazid determinations. Rifampicin in acidic ranges after six hours form precipitate that dissolved easily by mild shaking and the recorded absorbance decreased as pHs decreased which become stable after one week.

In method B, The effect of pH on the complex formation between Isoniazid or Rifampicin and NBS was studied in phosphate buffer solution of pH range 2.0-10.0. Fig. (6) depicts that the optimum pH range is (5.0 -8.0) and (4.0-7.5) for Isoniazid and Rifampicin respectively.

Effect of Time: In method, A Isoniazid is stable in pH 6.0 -7.5 for 6 days and Rifampicin is stable in neutral or slightly neutral medium until 6 days. In method, B the effect of time on the complex formation between Isoniazid and NBS was studied by measuring the absorbance at various time intervals before the reaction was stopped by adding Potassium iodide solution. Fig. (7) indicates that the suitable time is in between (5- 15) and above 5 minutes for Isoniazid and Rifampicin respectively.

Effect of Temperature: The effect of temperature is studied for the sample and the blank at different temperatures 25-60°C. Figure 8 show that the optimum temperature is below 35°C and below 30°C for Isoniazid and Rifampicin respectively.

Effect of Volume of Starch: The optimum volume of Starch which was sufficient to give a reasonable stable maximum absorbance with the Iodine liberated from 6.5 ml 2 x 10^{-4} mol L^{-1} NBS aqueous solution in 25 ml calibrated flask found to be above 2 ml 1% Starch solution as shown in Fig. (9).

Effect of Solvents: Different kinds of solvents are used as final dilution. Only distilled water was the suitable solvent used for this procedure. The immiscible organic solvents used were separate two layers with no change in color with the layer except in case of Anisidene. The miscible organic solvents used were hiding the color and another color with another absorbance appears.

Interference: No excipients present in the studied tablets and capsules used were interfere in both methods A and B. In method A for all studied drugs Urea, Sodium oxalate, Camphor, Glucose, Lactose, Sucrose, Ascorbic acid, Ethambutol dihydrochloride were not interfere. The results indicate that in presence of 10 mg ml^{-1} of them they don't interfere (absorbance changes by ± 2.0% is not interference). For Rifampicin; Hydrazine dichloride, Pyridoxine (vit. B_6) were not interfere. The results indicate that in presence of 10 mg ml^{-1} of them they do not interfere (absorbance changes by ± 2.0% is not interference). EDTA was slightly interfere with Rifampicin and Isoniazid by recoveries of 88.8 % and 25.9% respectively the previous results obtained by using 1 mg ml^{-1} excess of them. Hydrazine dichloride was slightly interfere with Isoniazid by recoveries of 77.7 % also Pyridoxine (vit. B_6) was strongly interfere with Isoniazid. Because that the peaks of Pyrazinamid and Isoniazid were not differ enough in the electronic spectra and there's additional peaks of Rifampicin in there determination region there are difficult to determine the two drugs simultaneously or in the presence of Rifampicin. But Rifampicin where determined at 474 nm was not interfere with Isoniazid or Pyrazinamide as shown in Fig. (3).

In method B Camphor, Hydrazine dichloride, Pyridoxine (vit. B_6) and Ascorbic acid were strongly interfering where the absorbencies become zero at any drug concentration. Glucose, Lactose, Sucrose and EDTA were slightly interfere with Isoniazid by recoveries of 77.7 %, 88.8 %, 19.9% and 33.7 % respectively for Isoniazid and for Rifampicin by recoveries of 80%, 93.5%, 40% and 60% respectively. The previous results obtained by using 1 mg ml^{-1} excess of them. Urea, Sodium oxalate, Ethambutol dihydrochloride and Pyrazinamide were not interfering. The results indicate that in presence of 10 mg ml^{-1} of them they do not interfere (absorbance changes by ± 2.0% is not interference).

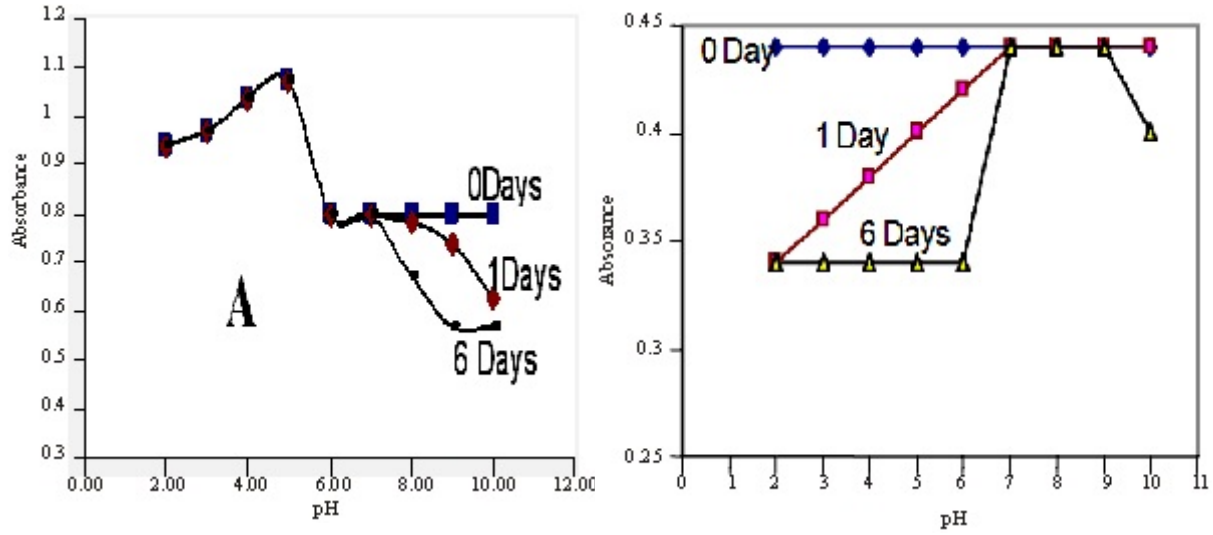


Fig. 5: Effect of pH and time period on A) Isoniazid, and B) Rifampicin.

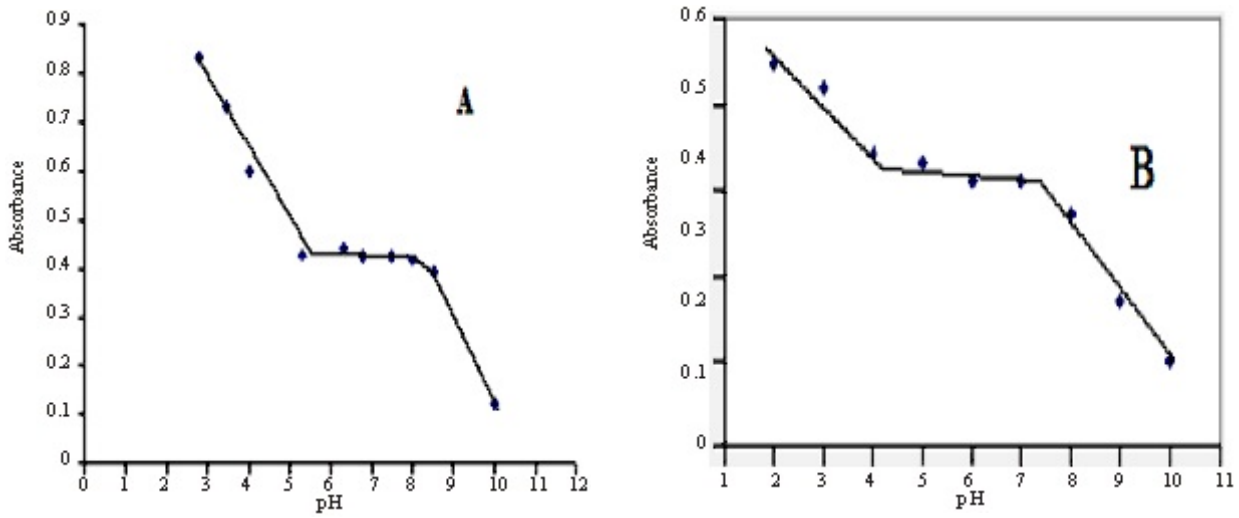


Fig. 6: Effect of different pHs on the absorbance for A) Isoniazid and B) Rifampicin. Final concentrations of Isoniazid and Rifampicin were 1.64 and 9.87 (μg ml^{-1}) using phosphate buffer.

Analytical Data: Beer's law limits of (2 – 42) and (0.8 - 65.38) μg ml^{-1} in method A and of (0.1 -3.4) and (0.5 - 15.5) μg ml^{-1} in method B for Isoniazid and Rifampicin respectively with a correlation coefficient ≤ 0.998, molar absorptivity, sandell sensitivity, regression equation and standard deviation obtained by linear least square treatment of the results are given in Table (1). For more accurate results, Ringbom optimum concentration recorded in Table (1). Recoveries and range of error percentages are also calculated and recorded in Tables (2 and 3).

For Pharmaceutical Preparations: The proposed method was successfully applied to various dosage forms, viz. tablets (Isocid, Isocid fort) and viz capsules (Riozid and Rimactazid both 300 mg Rifampicin + 150 mg Isoniazid). The results are recorded in Tables (2 and 3) compared statistically with the official U.S.P. HPLC methods reveal that the recoveries are in the range (94.03-103.34) for method A and (98.08 – 100.99) for method B reflects high accuracy, in addition to the high precision indicated by very low values of relative standard deviations. The performance of the proposed methods was assessed by calculation of t and f values compared with the official method. Mean values obtained in the students showed the absence of systematic errors in the method.

For Biological Fluids: Different serial dilutions of biological fluid were carried out and the previous

Table 1: Characteristics of the calibration graph for the developed methods (A and B).

Parameter	Units	Method A		Method B	
		Isoniazid	Rifampicin	Isoniazid	Rifampicin
Slope	ml $\mu g^{-1}cm^{-1}$	0.0288	0.0167	-0.283	-0.064
Intercept	-	0.0137	0.004	0.97	1.017
correlation coefficient	-	0.999	0.999	1.01	0.988
λ_{max}	ηm	264	474	572	572
Molar absorptivity	L $mol^{-1}cm^{-1}$	$3.97x\ 10^{3}$	$13.73\ x\ 10^{3}$	$38.91\ x\ 10^{-3}$	$53.1\ x\ 10^{-3}$
Specific absorptivity (a)	ml $g^{-1}cm^{-1}$	$28.95\ x\ 10^{-3}$	$16.7\ x\ 10^{3}$	$2.83\ x\ 10^{-5}$	$64\ x\ 10^{3}$
Beer's range	$\mu g\ ml^{-1}$	2--42	0.822- 65.38	0.1 - 3.4	0.5 - 15.9
Ringbom range	$\mu g\ ml^{-1}$	-	-	0.27 - 2.81	1.51 - 12.58
Quantification limit	$\mu g\ ml^{-1}$	-	-	1.77	8.91
Sandell sensitivity	$\mu g\ cm^{-2}$	$1.22\ x10^{2}$	$17.31\ x\ 10^{2}$	$9.72\ x10^{2}$	$29.13\ x\ 10^{3}$

Table 2: Accuracy and precision for Isoniazid and Rifampicin determinations by method A.

Active substance	Sample	Taken ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)$^{\bullet}_{[\bullet\bullet]}$	Recovery (%)$^{\bullet}_{[\bullet\bullet]}$	± S.D. ($\mu g\ ml^{-1}$)$^{\bullet}_{[\bullet\bullet]}$	t-value $^{\bullet}_{[\bullet\bullet]}$	F – test
Isoniazid	Isocid (50 mg) tablests	5.4	5.46 [5.44]	99.64 [99.27]	0.01 [0.02]	2.50 [1.67]	2.25
		13.7	13.80 [13.8]	100.67 [100.66]	0.08 [0.1]	2.01 [2.5]	1.69
		27.4	28.00 [27.5]	102.12 [100.29]	0.30 [0.21]	4.83 [2.38]	2.04
		41.1	40.92 [42]	99.47 [102.09]	0.60 [0.5]	0.92 [5]	1.44
			Average	100.47 [100.58]			
	Isocid (200 mg) tablests	5.4	5.41 [5.42]	98.72 [98.91]	0.03 [0.07]	5.00 [0.36]	5.44
		13.7	13.58 [13.69]	99.05 [99.85]	0.11 [0.09]	3.64 [0.28]	1.49
		27.4	27.18 [27.25]	99.12 [99.38]	0.46 [0.22]	1.30 [0.57]	4.37
		41.1	39.50 [40.5]	96.01 [98.44]	0.86 [0.5]	4.77 [2.5]	2.96
			Average	98.23 [99.15]			
Rifampicin	Riozid (300 mg) Capsoules	8.2	8.30 [8.31]	100.97 [101.09]	0.200 [0.14]	1.13 [1.61]	2.04
		16.4	17.00 [16.8]	103.34 [102.13]	0.500 [0.51]	2.80 [2.45]	1.04
		32.9	33.10 [33.2]	100.55 [100.85]	1.400 [0.73]	0.18 [1.06]	3.68
		65.8	67.80 [66.1]	102.98 [100.39]	1.700 [1.4]	2.85 [0.18]	1.47
			Average	101.96 [101.12]			

Table 2: Continued

	Rimactazid (300 mg) Capsules	8.2	8.10 [8.2]	98.54 [99.76]	0.260 [0.17]	1.06 [0.29]	2.34
		16.4	16.00 [16.2]	97.26 [98.48]	0.500 [0.3]	2.20 [0.83]	2.78
		32.9	30.95 [31.5]	94.02 [95.69]	1.200 [0.8]	4.27 [4.34]	2.25
		65.8	63.00 [64.6]	95.69 [98.12]	1.800 [1.3]	3.97 [2.69]	1.92
			Average	96.38 [98.01]			

●Developed method
[●●] Official method

Table 3: Accuracy and precision for Isoniazid and Rifampicin determinations by method B.

Active substance	Sample	Taken ($\mu g\ ml^{-1}$)	Found ($\mu g\ ml^{-1}$)$^{\bullet}_{[\bullet\bullet]}$	Recovery (%)$^{\bullet}_{[\bullet\bullet]}$	± S.D. ($\mu g\ ml^{-1}$)$^{\bullet}_{[\bullet\bullet]}$	t-value$^{\bullet}_{[\bullet\bullet]}$	F – test
Rifampican	Rimactan (300 mg)	1.6	1.65 [1.65]	100.61 [100.91]	0.006 [0.011]	4.25 [5.22]	1.21
	capsules	3.2	3.32 [3.31]	100.91 [100.61]	0.016 [0.02]	3.13 [3.75]	1.56
		9.8	9.90 [9.91]	100.30 [100.41]	0.045 [0.05]	2.78 [0.5]	1.23
		13.1	13.20 [13.30]	100.23 [100.99]	0.080 [0.07]	1.25 [3.93]	1.31
			Average	100.51 [100.73]			
Isocid (50 mg)		0.54	0.55 [0.55]	99.64 [100.37]	0.006 [0.009]	2.50 [1.66]	2.25
	tablets	1.09	1.11 [1.099]	101.19 [100.18]	0.008 [0.011]	3.13 [4.32]	1.89
		1.6	1.64 [1.648]	100.12 [100.49]	0.009 [0.019]	6.11 [5]	4.45
		2.7	2.75 [2.739]	100.37 [99.99]	0.028 [0.022]	2.67 [0.59]	1.62
			Average	100.33 [100.26]			
	Isocid fort (200 mg)	0.54	0.55 [0.54]	99.45 [98.91]	0.008 [0.007]	1.56 [0.71]	1.31
	tablets	1.09	1.09 [1.07]	99.36 [97.53]	0.009 [0.018]	2.77 [1.39]	4.00
		1.6	1.62 [1.66]	98.47 [101.10]	0.007 [0.015]	1.76 [8]	4.48
		2.7	2.71 [2.73]	98.90 [99.5]	0.029 [0.018]	0.86 [1.14]	2.59
			Average	99.05 [99.26]			

●Developed method
[●●]Official method

Table 4: Accuracy and precision for Isoniazid determinations in urine and plasma samples by method A.

Sample	Taken (μg ml^{-1})	Found (μg ml^{-1})●[●●]	Recovery (%)●[●●]	± S.D. (μg ml^{-1})●[●●]	t-value●[●●]	F – test
Urine 1	0.54	0.55 [0.55]	100.37 [100.18]	0.02 [0.009]	0.38 [1.66]	4.94
	1.3	1.36 [1.35]	99.20 [98.47]	0.05 [0.022]	0.70 [2.27]	5.17
	2.7	2.72 [2.74]	99.02 [99.93]	0.03 [0.061]	2.92 [0.41]	4.14
	4.1	4.10 [4.1]	99.68 [99.73]	0.10 [0.12]	0.33 [0.062]	1.44
		Average	99.57 [99.58]			
Urine 2	0.548	0.54 [0.541]	98.36 [98.72]	0.01 [0.007]	4.00 [0.71]	1.96
	1.37	1.38 [1.4]	100.66 [102.11]	0.03 [0.03]	0.60 [2.5]	1.44
	2.74	2.71 [2.73]	98.83 [99.56]	0.05 [0.036]	2.22 [0.0]	1.56
	4.11	4.06 [4.2]	98.69 [102.09]	0.06 [0.068]	2.45 [3.68]	1.52
		Average	99.13 [100.62]			
Plasma 1	0.54	0.56 [0.544]	102.19 [99.34]	0.01 [0.012]	4.06 [0.29]	2.25
	1.3	1.38 [1.4]	100.66 [102.12]	0.05 [0.03]	0.33 [2.5]	2.25
	2.7	2.71 [2.77]	98.83 [101.02]	0.08 [0.052]	1.25 [1.92]	2.37
	4.1	4.08 [4.11]	99.17 [99.93]	0.11 [0.065]	0.78 [0.42]	2.86
		Average	100.21 [100.6]			
Plasma 2	0.54	0.56 [0.542]	101.28 [98.91]	0.01 [0.011]	2.85 [0.125]	2.46
	1.3	1.39 [1.36]	101.39 [99.20]	0.03 [0.042]	1.42 [0.595]	2.25
	2.7	2.76 [2.75]	100.66 [100.29]	0.06 [0.052]	0.40 [0.961]	1.42
	4.1	4.09 [4.1]	99.42 [99.66]	0.08 [0.07]	0.75 [0.0]	1.31
		Average	100.68 [99.51]			

●Developed method
[●●]Official method

Table 5: Accuracy and precision for Rifampicin determinations in urine and plasma samples by method A.

Sample	Taken (µg ml^{-1})	Found (µg ml^{-1})$^{\bullet}_{[\bullet\bullet]}$	Recovery (%)$^{\bullet}_{[\bullet\bullet]}$	± S.D. (µg ml^{-1})$^{\bullet}_{[\bullet\bullet]}$	t- value$^{\bullet}_{[\bullet\bullet]}$	F –test
Urine 1	8.2	8.20 [8.14]	99.76 [99.02]	0.110 [0.16]	0.23 [1.25]	2.12
	16.4	16.50 [16.49]	100.30 [100.24]	0.170 [0.18]	0.88 [2.64]	1.12
	32.9	33.11 [32.99]	100.55 [100.21]	0.400 [0.32]	0.63 [0.78]	1.56
	65.8	65.20 [66.1]	99.03 [100.39]	0.530 [0.46]	3.11 [0.54]	1.33
		Average	99.91 [99.96]			
Urine 2	8.2	8.15 [8.13]	99.15 [98.93]	0.120 [0.16]	1.25 [1.38]	1.78
	16.4	16.35 [16.26]	99.39 [98.84]	0.210 [0.17]	1.07 [0.59]	1.53
	32.9	32.40 [32.4]	98.42 [98.42]	0.350 [0.27]	4.29 [4.54]	1.06
	65.8	65.00 [64.9]	98.72 [98.57]	0.610 [0.52]	3.52 [5.29]	1.26
		Average	98.92 [98.69]			
Plasma 1	82.2	81.9 [81.8]	99.64 [99.51]	1.30 [1.7]	0.38 [0.59]	1.71
	164.5	165.0 [165.5]	100.30 [100.61]	2.10 [2.5]	0.71 [2.5]	1.42
	329.2	332.0 [335]	100.85 [101.76]	3.50 [3.6]	1.43 [4.24]	1.06
	658.4	661.0 [664]	100.39 [100.85]	6.40 [5.7]	0.94 [1.75]	1.26
		Average	100.30 [100.68]			
Plasma 2	82.2	81.1 [820]	98.66 [99.75]	1.40 [1.5]	1.79 [0.33]	1.15
	164.5	163.7 [165]	99.51 [100.3]	1.70 [2.4]	1.03 [2.08]	1.99
	329.2	325.0 [332]	98.72 [100.85]	3.20 [3.6]	3.91 [2.15]	1.27
	658.4	652.0 [650]	99.03 [98.72]	4.50 [6.6]	3.67 [3.79]	2.15
		Average	98.98 [99.91]			

●Developed method
[●●]Official method

Table 6: Accuracy and precision for Isoniazid determinations in urine and plasma samples by method B.

Sample	Taken (µg ml^{-1})	Found (µg ml^{-1})●[●●]	Recovery (%)●[●●]	± S.D. (µg ml^{-1})●[●●]	t- value●[●●]	F –test
Urine 1	0.54	0.55	100.18	0.009	1.94	1.27
		[0.541]	[98.72]	[0.008]	[0.94]	
	1.0	1.08	98.45	0.020	2.50	1.10
		[1.04]	[94.8]	[0.021]	[4.76]	
		0.050	1.25	2.82	1.6	1.65100.30
		[1.65]	[100.6]	[0.084]	[1.19]	
	2.7	2.73	99.71	0.060	0.50	1.44
		[2.73]	[99.85]	[0.05]	[0.07]	
		Average	99.66			
			[98.49]			
Urine 2	0.54	0.55	100.37	0.007	2.85	1.31
		[0.56]	[101.28]	[0.008]	[3.44]	
	1.0	1.10	100.27	0.030	0.00	2.78
		[1.11]	[101.19]	[0.018]	[4.17]	
	1.6	1.66	101.22	0.050	2.00	1.00
		[1.63]	[99.57]	[0.05]	[1.15]	
	2.7	2.76	100.73	0.040	2.50	1.78
		[2.77]	[101.09]	[0.03]	[2.96]	
		Average	100.65			
			[100.78]			
Plasma 1	0.54	0.55	99.64	0.004	2.50	5.06
		[0.56]	[102.01]	[0.009]	[4.17]	
	1.0	1.08	98.45	0.031	1.61	1.32
		[1.03]	[93.89]	[0.027]	[4.63]	
	1.6	1.64	99.94	0.050	0.95	1.44
		[1.65]	[100.61]	[0.06]	[1.66]	
	2.7	2.76	100.73	0.060	1.67	1.36
		[2.8]	[102.19]	[0.07]	[2.34]	
		Average	99.69			
			[99.67]			
Plasma 2	0.54	0.55	100.18	0.008	2.19	1.31
		[0.54]	[98.54]	[0.007]	[1.43]	
	1.0	1.09	99.27	0.036	0.76	4.00
		[1.09]	[99.27]	[0.018]	[1.25]	
	1.6	1.66	101.22	0.050	2.00	1.44
		[1.64]	[99.82]	[0.06]	[1.13]	
	2.7	2.80	102.19	0.040	5.00	3.06
		[2.81]	[102.55]	[0.07]	[2.67]	
		Average	100.72			
			[100.05]			

●Developed method

[●●]Official method

Table 7: Accuracy and precision for Rifampicin determinations in urine and plasma samples by method B.

Sample	Taken (µg ml^{-1})	Found (µg ml^{-1})●[●●]	Recovery (%)●[●●]	± S.D. (µg ml^{-1})●[●●]	t-value●[●●]	F – test
Urine 1	1.6	1.63 [1.65]	99.39 [100.6]	0.01 [0.009]	0.83 [5]	1.00
	3.2	3.27 [3.3]	99.39 [100.3]	0.04 [0.021]	1.87 [2.38]	3.63
	9.8	9.80 [9.85]	99.29 [99.79]	0.09 [0.084]	1.39 [1.488]	1.15
	13.1	13.00 [13.1]	98.71 [99.47]	0.10 [0.15]	4.00 [1.5]	2.25
		Average	99.19 [100.04]			
Urine 2	1.6	1.65 [1.63]	100.61 [99.39]	0.01 [0.008]	4.25 [0.63]	1.56
	3.2	3.32 [3.29]	100.91 [100]	0.03 [0.018]	1.67 [1.39]	2.78
	9.8	10.00 [9.95]	101.32 [100.82]	0.09 [0.05]	4.17 [2.5]	3.24
	13.1	13.40 [13.21]	101.75 [100.3]	0.13 [0.2]	4.62 [0.25]	2.37
		Average	101.15 [100.13]			
Plasma 1	1.6	1.65 [1.65]	100.61 [100.61]	0.02 [0.009]	2.83 [5]	2.78
	3.2	3.35 [3.32]	101.82 [100.91]	0.03 [0.027]	4.03 [3.70]	1.32
	9.8	10.10 [9.9]	102.33 [100.30]	0.09 [0.06]	6.94 [0.00]	2.25
	13.1	13.60 [13.6]	103.27 [103.26]	0.20 [0.25]	5.50 [4.1]	1.56
		Average	102.01 [102.01]			
Plasma 2	1.6	1.63 [1.63]	99.39 [99.57]	0.02 [0.007]	0.42 [0.357]	6.61
	3.2	3.21 [3.27]	97.56 [99.39]	0.04 [0.018]	6.25 [1.388]	4.00
	9.8	9.67 [9.8]	97.97 [99.29]	0.09 [0.098]	5.00 [2.55]	1.19
	13.1	13.00 [13]	98.71 [98.71]	0.16 [0.12]	2.50 [3.958]	1.78
		Average	98.41 [99.24]			

●Developed method
[●●]Official method

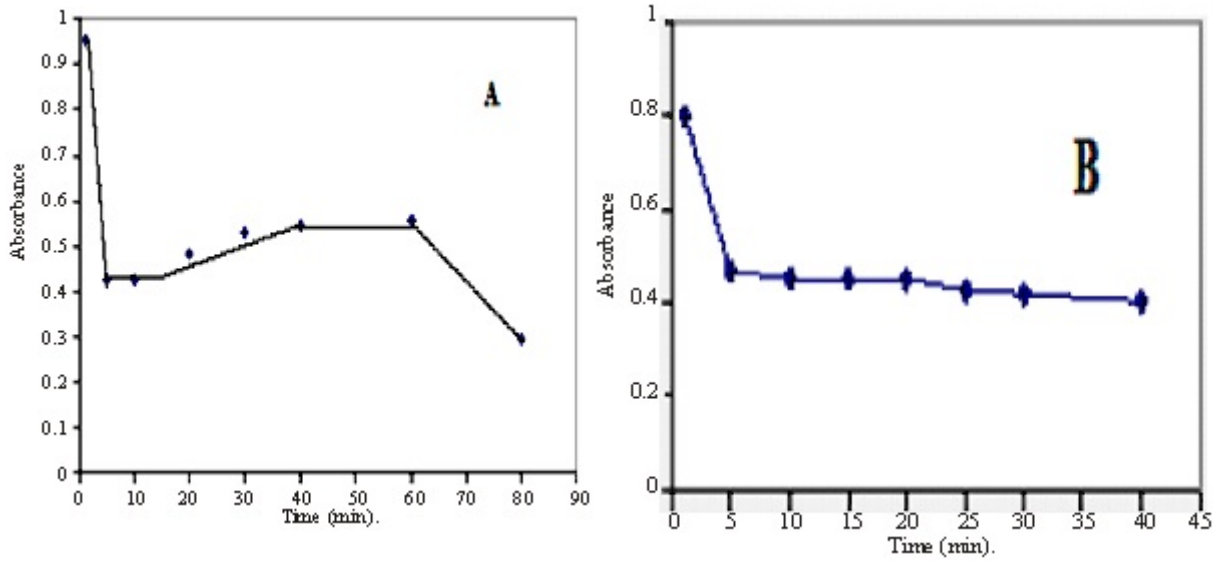


Fig. 7: Effect of time on the absorbance for A) Isoniazid and B) Rifampicin. Final concentrations of Isoniazid and Rifampicin were 1.64 and 9.87 (μg ml^{-1}).

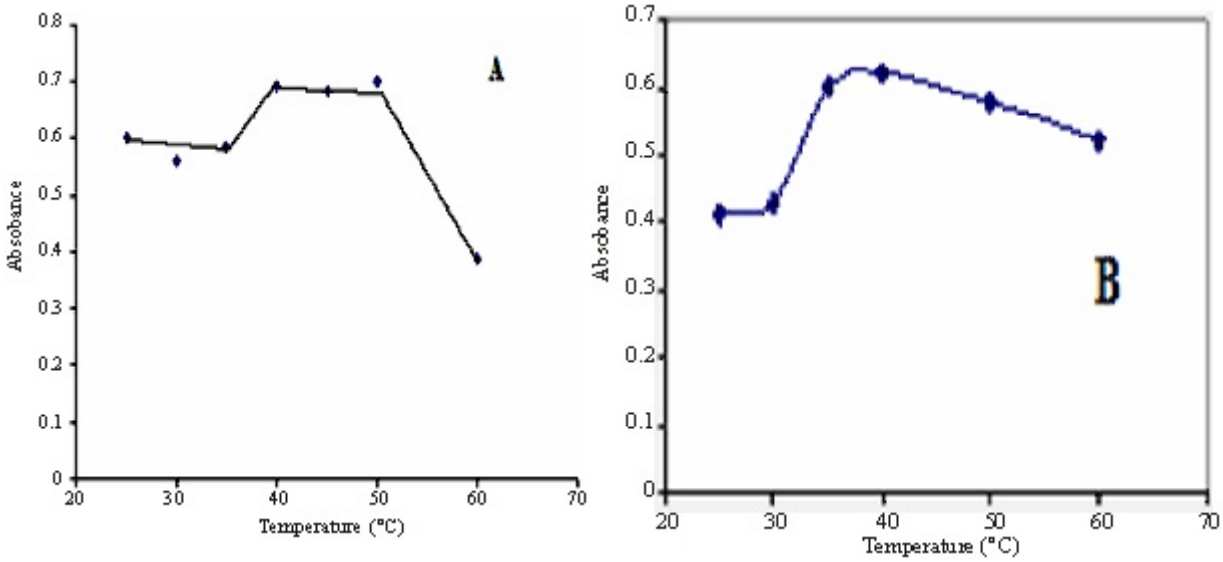


Fig. 8: Effect of temperature on the absorbance for A) Isoniazid and B) Rifampicin. Final concentrations of Isoniazid and Rifampicin were 1.64 and 9.87 (μg ml^{-1})

procedures were applied without adding the drug. In method A Up to (1:1000) final dilution, the region of determination becomes loudy reflects high degree of interference. In (1:10,000) final dilution, the previous general procedure was applied successfully. In method B Up to (1:1000) final dilution, the resulted absorbance becomes zero reflects high degree of interference. In (1:10,000) final dilution, the previous general procedure was applied successfully. By precipitate proteins by saturated Sodium chloride solution then centrifugation of the precipitated proteins and other amorphous elements and cells. In method A Up to (1:50) final dilution, the region of determination becomes also loudly reflects slightly degree of interference. In (1:100) final dilution, the previous general procedure was applied successfully and the results were tabulated in Table (3). For Rifampicin there was slightly peaks in the detection region which not interfere with the proposed method the previous general procedure was applied successfully. In method B Up to (1:100) final dilution, the resulted absorbance becomes zero reflects high degree of interference. In (1:1000) final dilution,

the previous general procedure was applied successfully and the results were tabulated.

In method A, for urine samples average recoveries in the ranges (98.36% - 102.11%), and (98.42% - 100.55%), respectively. T-values of (0.38 - 2.5) and (0.23 - 4.29) for Isoniazid and Rifampicin respectively. For plasma samples average recoveries in the ranges (99.17% - 102.19%) and (98.66% - 100.85%) for Isoniazid and Rifampicin respectively. T-values of (0.33-4.06) and (0.38 - 3.91) for Isoniazid and Rifampicin respectively (Tables 4 and 5).

In method B For urine average recoveries in the ranges (98.45%-101.22 %) and (98.71% - 101.75%) for Isoniazid and Rifampicin respectively. T-values of (0.0- 2.85) theoretical one at 95% confidence level indicate insignificant differences between the measured and real concentrations.

For Plasma samples average recoveries in the ranges (98.45 - 102.19 %) and (97.56-103.27%) for Isoniazid and Rifampicin respectively. T-values of (1.0 - 5.00) and (0.42-6.25) for Isoniazid and Rifampicin respectively,the results tabulated in Tables (6 and 7). All were less than the theoretical one at 95% confidence level indicate insignificant differences between the measured and real concentrations. The obtained real concentrations present in the biological fluids compared well with those obtained using U.S.P. HPLC methods [2] and assisted by applying F-test which less than the theoretical one at 95% confidence level confirms high precision and accuracy.

Conclusion: The proposed spectrophotometric methods have been applied successfully for the analysis of the drugs (Isoniazid and Rifampicin) in pure, dosage forms and biological fluids. The developed methods would be simpler, highly sensitive, low reagent consumption, no interference and achieve higher accuracy and precision compared to previously published methods.

REFERENCES

1. European pharmacopoeia, fourth ed. 2002. Council of Europe 1404.
2. United States Pharmacopeoia, National Formulary (USP. NF), The Official Compenia of Standards, Assian edition, 2007, 3079 and 3128.
3. Jawetz, Melnick and Adelbergs 1998. Medical microbiology, 21: 171.
4. Guermouche, S. and M.H. Guermouche, 2004. J. chromatogr. sci., 42(5): 250.
5. Aït Moussa, L., C.E. Khassouania, R. Soulaymania, M. Janab, G. Cassanasc, R. Alricd and B. Hüed, 2005. J. Chromatography B, 5: 181.
6. Wang, Y., Xin, 1998. Yaowu Fenxi Zazhi, 8(3): 181.
7. Carlina, A., N. Gregoryb and J. Simmonsc, 1998. J. Pharmaceutical and Biomedical Analysis, 17: 885.
8. Safavi, A., M. A. Karimi1 and M.R. Hormozi, 2004. II Farmaco, 59: 481.
9. Safavi, A., Mohammad Ali Karimi and Mohammad Reza Hormozi Nezhad, 2003. J. Pharmaceutical and Biomedical Analysis, 30: 1499.
10. Maria, S., M. Quintinoa and Lúcio Angnes, 2006. J. Pharmaceutical and Biomedical Analysis, 42: 400.
11. Beltagi, A.M. and M.M. Ghoneim, 2004. Microchemical J., 77: 53.
12. Nagendra, P., H.S. Yathirajan and K.N. Mohona, 2002. J. Indian chem. Soc., 79(1): 75.
13. Sarwar, M., U.A. Malik, A Khan and C.S. Pakistan, 1989. Anal. lett., 22(4): 853.
14. Sulaiman, S.T. and D. Amin, 1983. Microchemical J., 28: 328.
15. Kulkarni, R.M., D.C. Bilehal and S.T. Nandibewoor, 2004. Anal.sci.Appr. 20(4): 743.
16. Nagaraja, P., K.C. Srinivasa Murthy and H.S. Yathirajan, 1996. Talanta, 43: 1075.
17. David, F., Bain, L. Dale Munday and J. Philip Cox, 1998. European J. Pharmaceutical Sciences, 7: 57.
18. Revankar, S.N., N.D. Desai and A.D. Bhatt, 2000. Indian drugs, 37(7): 323.
19. Yoe, Dong Liang, A.B., Jun, Feng Songa and Min Xua 2007. Spectrochimica Acta Part A, 67: 430.
20. School, Chem., X.L., Y.T. Zhang, H.Y. Ma and C.X. Zhang,, 2004. Fenxi-Kexue-Xuebao, 20(6): 604.
21. Alonso, Lomillo, M.A., J.M. Kauffmann, M.J. Arcos Martinez, 2003. Biosensors and Bioelectronics, 18: 1165.
22. Renê, H.T. Santos1, G. Neemias Santos1, José P.H. Alves1, Carlos A.B. Garcia, Luciane C.P. Romão1, E Maria Lara and P.M. Arguelho1, (in press) Bioelectrochemistry.
23. Galal, S.M, S.M. Blaih and M.E. Abdel-Hamid, 1992. Anal. Lett., 25(24): 725 .
24. Gandhi, T.P., A.A. Patel, R.R. Patel and V.C. Patel, 1978. Indian drugs, 16(1): 10.
25. European pharmacopeoia, 2002. Council of Europe, Strasburg, fourth edition, 1862.
26. Yoe, J.H. and A.L. Jones, 1944. Indust. Eng. Chem. (Anal., Ed.) 16: 111.
27. Barakat, M.Z. and M. Shaker, 1966. Analyst, 91: 466.