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Spectrophotometric Methods for Microdetermination of Some Important Antimycobacterial Drugs Using Iodine-Starch and Hydroquinone

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Abstract: Two simple, accurate and sensitive spectrophotometric methods are described for the determination of Isoniazid and Ethambutol dihydrochloride in their pure form, pharmaceutical preparations and biological fluids. Different reactions path ways were introduced in this work include the reaction of Isoniazid with Iodine - Starch solution (method A) and the reaction of Isoniazid and Ethambutol dihydrochloride with Hydroquinone (method B). The absorbances were measured after 10 min. at (λ_{max} = 572 nm; method A) and at (λ_{max} = 310 and 218 nm respectively for method B). Beer's low obeyed for both methods and relative standard deviation were found to be less than 1%. Also Student's t and f tests statistical treatments were applied for all results obtained in each procedure where all demonstrate acceptable accuracy and precision. This show the suitability of all procedures for safety accurate and simple use for quality control analysis.

Key words: Spectrophotometric · Isoniazid · Ethambutol dihydrochloride · Iodine-Starch · Hydroquinone · Pharmaceutical preparations · Body fluids

INTRODUCTION

Isoniazid is Isonicotinic acid hydrazide, INH [1] and Ethambutol dihydrochloride as antimycobacterial agents [2], Figure (1) are used for the treatment of T.B. which attracted special attention for their therapeutic importance [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 Ethambutol includes HPLC [17, 18], Electrometry [19], titrimetry [20] and Spectrophotometry [21, 22]. The published methods for the determination of each drug is relatively little.

The present work demonstrates complexation reactions of Isoniazid and Ethambutol dihydrochloride with Iodine- Starch solution and with Hydroquinone. The methods are applied for the assay of the cited drugs in pure forms, in pharmaceutical preparations and in biological fluids. The results obtained are favorably comparable with those of official methods [1].

Experimental

Apparatus: Shemadzu 160 A double beam self recording self recording spectrophotometer with 10 mm quartz cell.

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

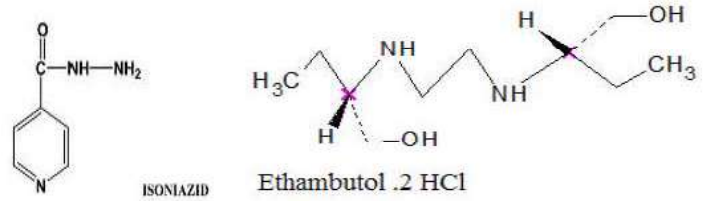

Fig. 1: Structure of the studied drugs

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MATERIALS

Isonicotinic hydrazide (Isoniazid) powder and Sodium Chloride purchased from Fluka company, Germany.

Ethambutol dihydrochloride supplied from Memphis company for pharmaceutical and chemical industries, Egypt.

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, Hydroquinone (HQ) and Sodium hydroxide (NaOH) analar purchased from Adwic. EDTA purchased from Win lab. U.K. Sodium oxalate purchased from Mallinckrodt chemicals. U.S.A. Acetonitrile and Methanol HPLC grade purchased from Scharlau chemie, Spain.

Market samples of Isocid 50 mg tablets and Isocid fort 200 mg tablets produced by CID Company Egypt. Riozid Capsules produced by Medical union pharmaceuticals (MUP), Abu-sultan, Ismailia, Egypt or Rimactazid Capsules produced by Novartis pharma, Egypt. Etibi 500 mg tablets produced by Memphis company, Egypt. All supplied from local market.

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

All solutions were freshly prepared. Double distilled water was used. Standard Isoniazid solutions with different concentrations $1x10^{-4}$M, $5x10^{-4}$M, $1x10^{-3}$M, $5x10^{-3}$ M. Standard $1x10^{-3}$M and $5x10^{-4}$M Ethambutol dihydrochloride aqueus solution. Solution A: $1x10^{-3}$ M Iodine solution prepared by mixing 0.0253 g Iodine with 0.5 g Potassium Iodide in 100 ml distilled water. Solution B: 1% Starch solution prepared by mixing 1 gm of soluble starch with 5 ml distilled water then completed to 100 ml boiled distilled water, shook till clear solution obtained and left to be cold before used. $2x10^{-4}$ M Iodine-Starch aqueous solution prepared by mixing 20 ml of solution A and 20 ml of solution B then completed to 100 ml distilled water in 100 ml measuring flask. $5x10^{-4}$M and $2x10^{-3}$M Hydroquinone (HQ) aqueous solution. 4% NaOH aqueous solution. Series of phosphate buffers pH 2.5 - 10. Saturated Sodium chloride solution.

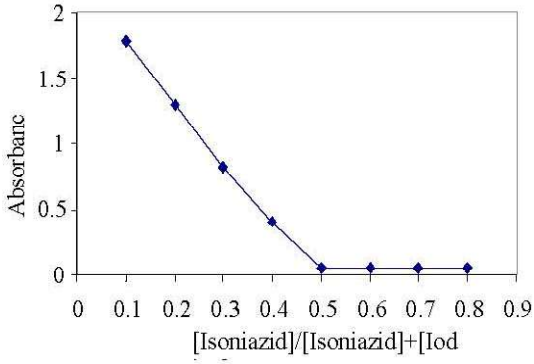


Fig. 2: Continuous variation of Isoniazid drug with Iodine

Procedures for Method A

Stoichiometry of the Formed Compound: Stoichiometry of the drug with Iodine was determined by applying the modified Job's Continuous variation method which performed by Vesburgh and Cooper [23], Figure 2. A series of solutions were prepared by mixing equimolar aliquots ($1x10^{-4}$M) of the standard Isoniazid solution and Iodine-Starch solution in different proportions while keeping the total molar concentration constant($1x10^{-4}$M) then left for 10 minutes. A plot of the absorbance values at wave length 572 nm of the resulting solutions versus the mole fraction of the drug manifests a minimum for the indirect methods at the expected molar ratio of the most stable complexes.

Procedure for Pharmaceutical Preparations: Twenty tablets (Isocid 50 mg and Isocid fort 200 mg) were weighted and ground to finely divided powder or the powder obtained from ten capsules (Riozid or Rimactazid 150 mg). An accurate weight of the powder contain 0.68 mg of Isoniazid was dissolved in to 50 ml distilled water to produce $1x10^{-4}$ M final concentration. The solution was then filtered off. 1-10 ml of the filtrate were transferred in to 25 ml calibrated flasks. In each calibrated flask 7.5 ml of Iodine-Starch solution was added. After 10 minutes, the absorbance's 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].

Procedure for Biological Fluids: 5 ml of biological fluid (urine or plasma) was spiked with 0.5- 25 mg of Isoniazid powder. 1 ml biological fluid (urine or plasma)

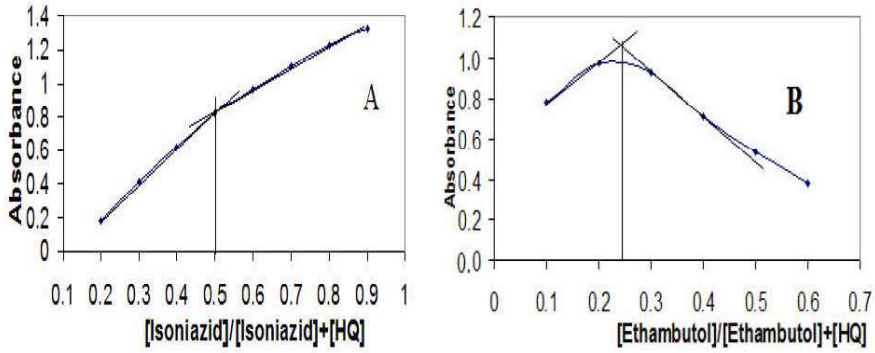


Fig. 3: Jobs variation plotting of A) Isoniazid and B) Ethambutol drug with HQ

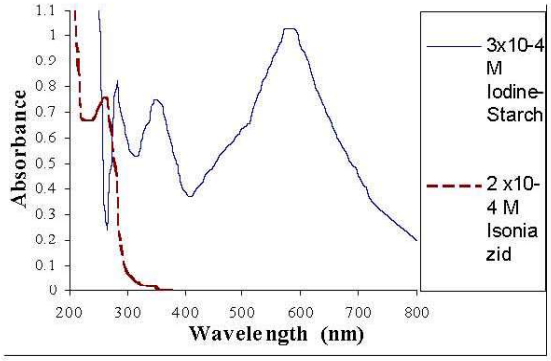


Fig. 4: Absorption spectra of Isoniazid and Iodine-Starch

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 prepared solution was transferred in to 25 ml volumetric flask then the general procedure was followed. 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 [2].

Procedures for Method B

Stoichiometry of the Formed Compound: Stoichiometry of the drug with Iodine was determined by applying the modified Job's Continuous variation method which performed by Vesburgh and Cooper [23] Figure 3. A series of solutions were prepared by mixing equimolar aliquots ($5x10^{-4}$M) of the standard (Isoniazid or Ethambutol dihydrochloride) solution and Hydroquinone in sodium hydroxide solution in different proportions while keeping the total molar concentration constant($5x10^{-4}$M) then left for 10 minutes. A plot of the absorbance values which measured at the same time against the reagent blank at wave length 310 nm of the resulting solutions versus the mole fraction of the drug manifests a maximum for the direct methods at the expected molar ratio of the most stable complexes.

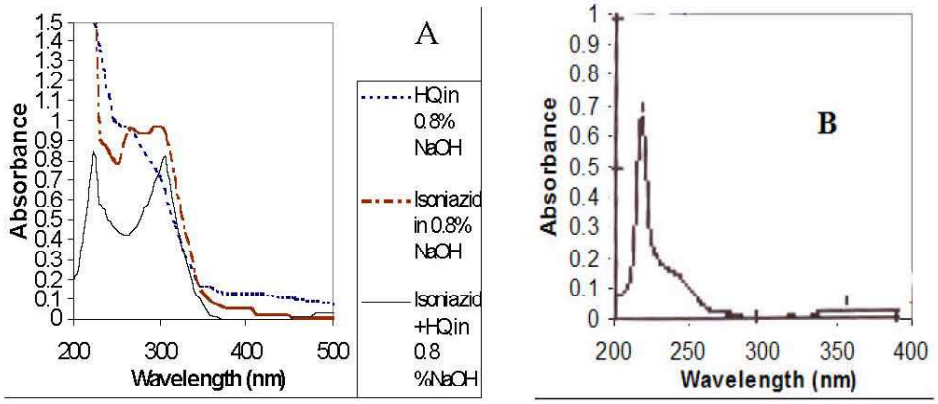


Fig. 5: Absorption spectra of A) Isoniazid and B) Ethambutol; Quinone products

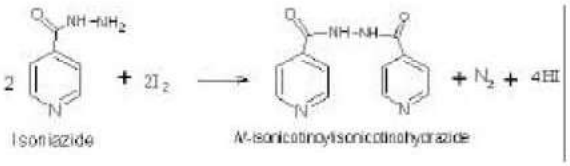
Procedure for Pharmaceutical Preparations: Twenty tablets were weighted and ground to finely divided powder. An accurate weights of the powder contain 6.8 mg, 13.87 of Isoniazid or Ethambutol dihydrochloride respectively, was dissolved in to 50 ml distilled water to produce $1x10^{-3}$ M final concentration. The solution was then filtered off. 1-6 ml were transferred in to 25 ml calibrated flasks. Each calibrated flask contains the reagent (1.5 and 8 ml of $1x10^{-3}$M HQ solution and 2 and 4 ml 4%NaOH solution) for Isoniazid and Ethambutol dihydrochloride respectively, then the absorbances were measured at wave length 310 and 218 nm respectively, against the reagent blank (prepared at the same time) Figure 5. In period from 10-25 minutes. Each measurement was repeated for five times and compared statistically with the official HPLC method [2].

Procedure for Biological Fluids: 5 ml of biological fluid (urine or plasma) was spiked with 0.5 - 50 mg of Isoniazid and 0.25-5.5 mg of Ethambutol dihydrochloride 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. 2.5 ml of the supernatant was transferred in to 25 ml volumetric flask then the general procedure was followed. 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].

RESULTS AND DISCUSSION

The different experimental parameters affecting the colour development were extensively studied to determine the optimal conditions for these procedures. In the present study, Figure 2 indicates that the stiochiometric ratio Iodine: Isoniazid is 1:1 which coincident with the formation of 1:1 charge transfer complex of the type (base) I_2 but the absence of any peaks of the formed complex above wavelength 270 nm if the Iodine-Starch completely consumed by the drug indicates another mechanism which iodination or oxidation.

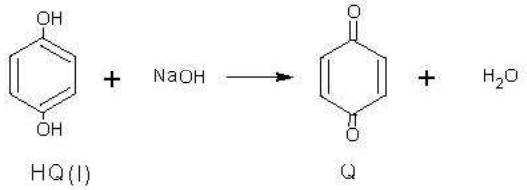
Canbäck [24] stated that Isoniazid consumed 4 aquivalents of iodine when oxidized and the reaction was in accordence with Equation 1.



Equation 1: The reaction pathway between Isoniazid and Iodine

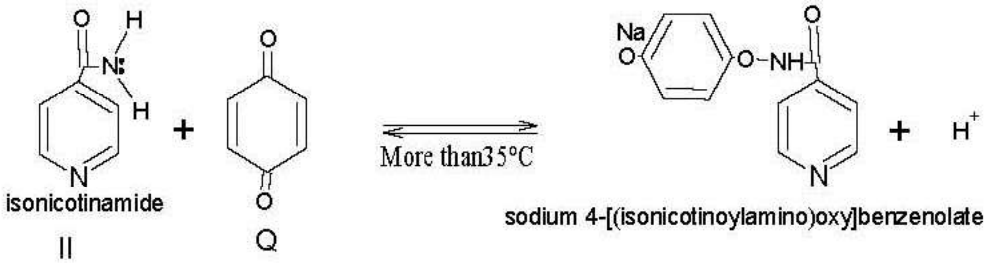
Isoniazid solution in high alkaline medium form Isonicotinamide (II) which have absorption spectra at 295 nm as shown in Figure 5 (A) [25].

Colourless hydroquinone (HQ) solution in alkaline medium transform to yellow 1,4 benzoquinone (Q) [26], by Equation 2.



Equation 2: The reversible conversion of Hydroquinone to Quinone

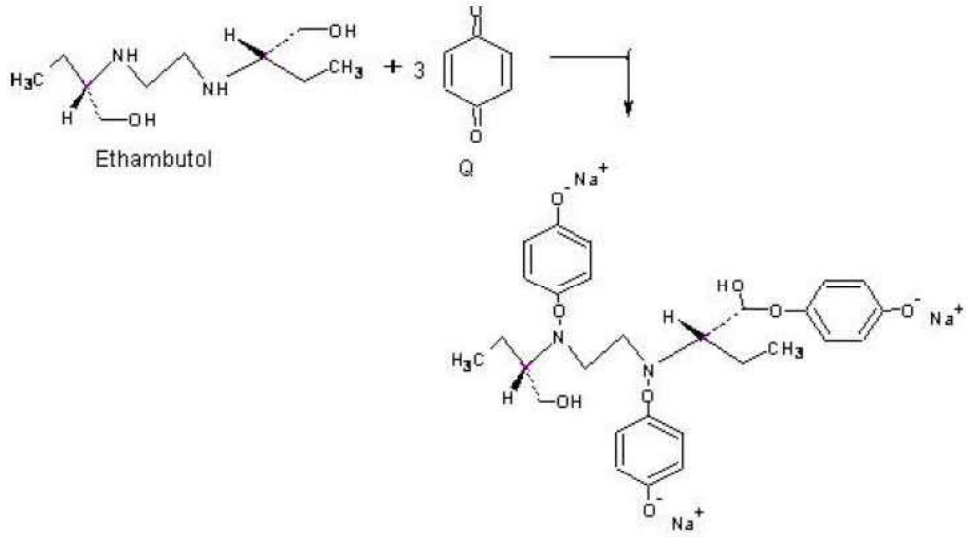
(Q) and (II) were react to form the studded product by stoichiometry (1:1), (Q: II) as shown in Figure 3, which represented by the proposed Equation 3.



Equation 3: The proposed reaction pathway between (II) and (Q).

This product has absorption spectra at 310 nm as shown in Figure 5 where the absorbance increase quantitatively as the concentration of the drug increase.

Etambutol dihydrochloride also react with Quinone to form the other studied product which have absorption spectra at wavelength 218 nm where the absorbance increase quantitatively as the concentration of the drug increases Figure 5.



Equation 4: The proposed reaction pathway between Ethambutol dihydrochloride and (Q).

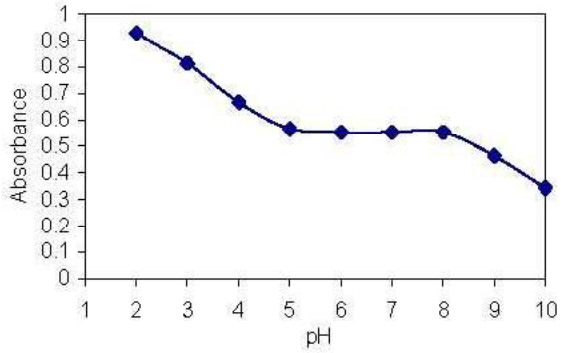


Fig. 6: Effect of different pHs on the absorbance in method A. Final concentration of the drug. was 2.74 ($\mu g\ ml^{-1}$).

Selection of Wavelength: The proper wavelength for the Iodine -Starch complex species was selected according to the absorption spectra shown in Figure 4 which indicates that the Iodine -Starch complex species gives maximum absorbance at wave length λ_{max} = 572 nm for method A. Also the absorption spectra shown in Figure 5, which indicates that the complex species gives maximum absorbance at wavelength λ_{max} =310 and 218 nm for Isoniazid and Ethambutol respectively, for method B.

Effect of pH: The effect of pH in method A on the complex formation between Isoniazid and Iodine was studied in different phosphate buffer media of pH range (2.0-10.0). Figure 6 depicts that the optimum pH range is (5.0 -8.0).

Effect of Volume of NaOH Solution: The optimum volume of 4% Sodium hydroxide that was sufficient to give a reasonable stable maximum absorbance with Hydroquinone and Isoniazid in 25 ml calibrated flask found to be above 2 and 4 ml for Isoniazid and Ethambutol dihydrochloride respectively, for method B as shown in Figure 7.

Sequence of Addition: Sequence of addition was determined by measuring the absorbance of all possible sequence of (The drug-Sodium hydroxide-Hydroquinone) in a total volume of 25ml measuring flask. The sequence of addition that gives the highest absorbance is (Hydroquinone-Sodium hydroxide-Isoniazid or Ethambutol dihydrochloride).

Effect of Time and Temperature: The effect of time on the complex formation between Isoniazid and Iodine was studied by measuring the absorbance at various time intervals Table 1 indicate that the suitable time after 10 minutes.

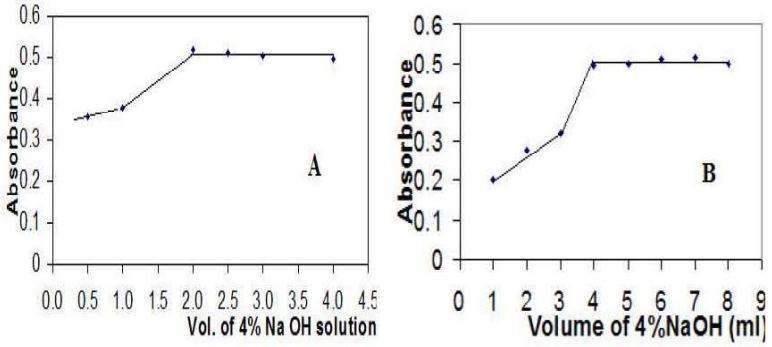


Fig. 7: Effect of different volumes of 4%NaOH solution on the absorbance of A) Isoniazid and B) Ethambutol dihydrochloride. Final drug concentration of Isoniazid and Ethambutol dihydrochloride was 43.88 and 5.54 (μg ml^{-1}) respectively.

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

			Method B	
		Method A		
Parameter	Units	Isoniazid	Ethambutol	Isoniazid
Time	Minutes	10	5-30	10-30
pH	-	5-8	-	-
slope	ml $\mu g^{-1}cm^{-1}$	-0.186	0.0924	0.0124
Intercept	-	1.09	-0.0101	-0.0635
correlation coefficient	-	0.991	0.998	0.995
λ_{max}	nm	572	218	310
Molar absorptivity (å)	L $mol^{-1}cm^{-1}$	27.2×10^{3}	25.6×10^{-3}	1.69×10^{-3}
Specific absorptivity (a)	mL $g^{-1}cm^{-1}$	1.86×10^{-5}	9.24 x 104	12.4×10^{3}
Beer's range	μg mL^{-1}	1.0 - 6.0	0.5-11.0	2 - 100
Ringbom range	μg mL^{-1}	1.12 - 5.11	2-12.5	1.47 - 89.12
Quantification limit	mg mL ^{1}	3.01	7.094	63.09
Sandell sensitivity	μg cm^{-2}	9.72 $x10^{3}$	20.01 $x 10^{2}$	10.01 $x10^{2}$

The effect of time on the complex formation between Isoniazid or Ethambutol dihydrochloride and Qinone was studied by measuring the absorbance of the sample against the blank at various time intervals. The absorbance became nearly constant in time interval (10- 30) and (5-30) minutes for Isoniazid and Ethambutol dihydrochloride respectively as shown in Table 1.

The effect of temperature was studied also for the sample and the blank at different temperatures 25-60°C. Table 1 show that the optimum temperature is below 35°C for both methods A and B.

Effect of Concentration of HQ Solution: The optimum Hydroquinone concentration which the maximum absorbance becomes constant was above $65x10^{-5}$ and 10 $x10^{-5}$ (mole L^{-1}) corresponding to Hydroquinone volume above 8 and 1.5ml 2 $x10^{-3}$ M (HQ) for Isoniazid and Ethambutol respectively as shown in Figure 8.

Effect of Solvents: Different kinds of solvents were used as final dilution. Only distilled water was the suitable solvent used for this procedure. The immiscible organic solvents used like Aniline, Benzene,Nitrobenzene, Chloroform,Tetrachloromethane,methylene dichloride, butanol were separate two layers with no change in colour with the another layer except in case of Anisidene. The miscible organic solvents used like Ethanol, methanol, Acetone, Acetonitrile, Dioxane, DMF, DMS were hide the colour of the Iodine - Starch solution for method A.

Interference: No excipients present in the tablets used were interfere in both methods. In method A Camphor, Hydrazine dichloride, EDTA and Ascorbic acid were strongly interfering. The absorbance becomes zero. Glucose, Lactose, Sucrose and Pyridoxine (vit. B_6) were slightly interfere with recoveries of 77.7%, 88.8%, 40.9%

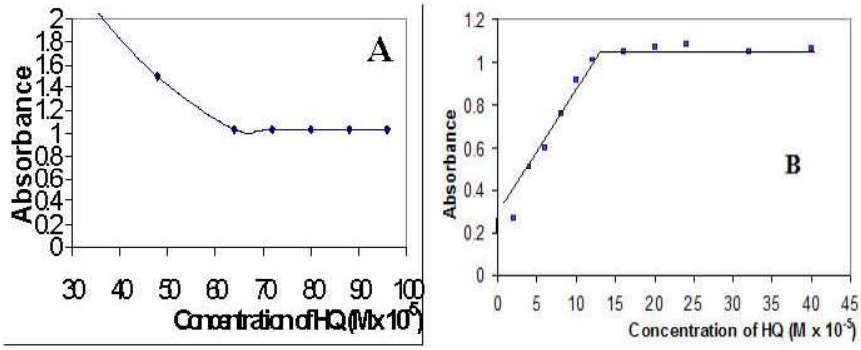


Fig. 8: Effect of different concentrations of HQ solutions on the absorbance of A) Isoniazid and B) Ethambutol dihydrochloride. Final drug concentration of Isoniazid and Ethambutol was 87.76 and 11.08 (μg ml^{-1}) respectively

Table 2: Accuracy and resection for Isoniazid determinations in urine and plasma samples* by method A.

Sample	Taken (mg ml^{-1})	Found (mg ml^{-1})•[••]	Recovery (%)•[••]	±S.D. (mg ml^{-1})•[••]	t- value•[••]	F - Test
Urine	1.64	1.64 [1.639]	99.70 [99.94]	0.01 [0.008]	2.28 [2.81]	1.56
	2.74	2.74 [2.76]	99.82 [100.73]	0.03 [0.018]	3.36 [3.54]	2.77
	3.83	3.80 [3.9]	99.22 [101.83]	0.05 [0.05]	1.03 [3.88]	1.00
	4.93	4.90 [4.81]	99.39 [97.57]	0.07 [0.06]	1.79 [3.87]	1.36
		Average	99.53 [100.02]			
Plasma	1.64	1.63 [1.648]	99.51 [100.49]	0.02 [0.009]	1.02 [5]	2.78
	2.74	2.73 [2.75]	99.64 [100.37]	0.03 [0.027]	3.65 [1.43]	1.32
	3.83	3.81 [3.9]	99.48 [101.83]	0.06 [0.06]	0.44 [3.24]	1.00
	4.93	4.85 [4.99]	98.38 [101.22]	0.05 [0.25]	5.00 [0.87]	0.04
		Average	99.25 [100.97]			

• Developed method

[••] Official method

* Average of three samples

and 133.7%, respectively, the previous results obtained by using 1 mg ml^{-1} excess of them. Urea, Sodium oxalate, Ethambutol dihydrochloride and Pyrazinamide not interfere. The result indicates that in presence of 10 mg ml^{-1} of them, they don't interfere (absorbance changes by ±2.0% is not interference). Rifampicin in neutral or slightly neutral medium tell 2 x 10^{-4}M 164.44 μg ml^{-1} (25 fold excess)was not interfere. In method B For all studied drugs Urea, Sodium oxalate, Camphor, Glucose, Lactose, Sucrose, Ascorbic acid, 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). Hydrazine dichloride, Pyridoxine (vit. B_6) and Rifampicin were strongly interfere. EDTA was slightly interfere with Isoniazid and Ethambutol dihydrochloride by recoveries of 123%, the previous result obtained by using 1 mg ml^{-1} excess of it. Pyrazinamide not interfere with Isoniazid till 25 fold excess and strongly interfere with Ethambutol dihydrochloride by its electronic spectra

Analytical Data: Beer's law limits in method A of (1.0 - 6.0) μg ml^{-1} and in method B of (2-100) and (0.5-11) μg ml^{-1} for Isoniazid and Ethambutol dihydrochloride respectively. with a correlation coefficient = 0.98, molar absorptivity, sandell sensitivity, regression equation and standard deviation obtained by linear least square treatment of the results are given in table1. For more accurate results, Ringbom optimum concentration recorded in Table 1.

For Pharmaceutical Preparations (Tablets): The proposed methods were successfully applied to various dosage forms, viz. Tablets (Isocid 50 mg, Isocid fort 200 mg and Etibi 500mg). Recoveries and relative standard deviation are aiso calculated and recorded in Tables 2 and 3 compared statistically with the official method[2] reveal that the recoveries are in the ranges of (99.02%-101.28%) in method A and (98.44 - 99.86)% and (97.31-100.73%) for Ethambutol dihydrochloride and Isoniazid respectively in

Table 3: Accuracy and precision for Isoniazid tablets and capsules determinations by method A.

Active Substance	Sample	Taken (μg ml^{-1})	Found (μg ml^{-1})•[••]	Recovery (%)•[••]	± S.D. (μg ml^{-1})•[••]	t- Value•[••]	F - test
Isoniazid	Isocid (50 mg) tablets	1.64	1.64 [1.648]	100.13 [100.48]	0.012 [0.019]	3.38 [5]	2.51
		2.74	2.79 [2.739]	101.95 [99.99]	0.025 [0.022]	1.82 [0.59]	1.29
		3.83	3.85 [3.847]	100.57 [100.46]	0.030 [0.035]	2.61 [1.81]	1.36
		4.93	4.99 [4.938]	101.28 [100.16]	0.038 [0.051]	2.85 [1.72]	1.80
			Average	100.98 [100.28]			
	Isocid fort(200mg) tablets	1.64	1.63 [1.639]	99.40 [99.94]	0.010 [0.015]	1.06 [4.83]	2.25
		2.74	2.74 [2.726]	100.17 [99.50]	0.018 [0.018]	4.24 [1.14]	1.00
		3.83	3.79 [3.814]	99.02 [99.60]	0.026 [0.025]	2.70 [0.77]	1.08
		4.93	4.89 [4.927]	99.20 [99.95]	0.037 [0.043]	4.02 [1.43]	1.35
			Average	99.45 [99.75]			
	Riozid (150mg) Capsules	1.64	1.61 [1.62]	98.17 [98.78]	0.008 [0.008]	4.95 [3.13]	1.00
		2.74	2.71 [2.7]	98.91 [98.54]	0.028 [0.017]	5.82 [5.08]	2.71
		3.83	3.79 [3.75]	98.96 [97.91]	0.032 [0.035]	2.39 [5.17]	1.20
		4.93	4.88 [4.85]	98.99 [98.38]	0.060 [0.07]	2.92 [1.89]	1.36
			Average	98.75 [98.4]			
	Rimactazid (150 mg) Capsules	1.64	1.62 [1.615]	98.78 [98.47]	0.009 [0.005]	2.50 [2.5]	3.24
		2.74	2.72 [2.71]	99.27 [98.91]	0.021 [0.014]	5.56 [4.38]	2.25
		3.83	3.80 [3.78]	99.22 [98.69]	0.040 [0.03]	5.95 [3.53]	1.78
		4.93	4.80 [4.82]	97.36 [97.77]	0.060 [0.05]	4.00 [4.14]	1.44
			Average	98.66 [98.46]			

• Developed method

[••] Official method

Table 4: Accuracy and presecion for Isoniazid and Ethambutol tablets determinations by method B.

Active Substance	Sample	Taken (μg ml^{-1})	Found (μg ml^{-1})•[••]	Recovery (%)•[••]	± S.D. (μg ml^{-1})•[••]	t-value•[••]	F - test
Isoniazid	Isocid (50 mg) tablets	10.97	10.98 [11]	100.09 [100.27]	0.12 [0.24]	1.67 [1.15]	4.00
		21.94	22.00 [22.2]	100.27 [101.18]	0.18 [0.22]	1.39 [4.55]	1.49
		43.88	44.20 [44.2]	100.73 [100.73]	0.20 [0.35]	6.25 [2.86]	3.06
		65.82	66.00 [66.8]	100.27 [101.49]	0.25 [0.563]	2.00 [3.99]	5.07
			Average	100.34 [100.92]			
	Isocid fort(200mg) tablets	10.97	10.80 [10.82]	98.45 [98.63]	0.10 0.21	2.50 [0.83]	4.41
		21.94	21.50 [21.7]	97.99 [98.91]	0.18 0.18	5.56 [1.39]	1.00
		43.88	42.70 [43]	97.31 [97.99]	0.42 0.30	5.95 [6.66]	1.96
		65.82	65.00 [65.2]	98.75 [99.06]	0.50 0.46	4.00 [3.80]	1.18
			Average	98.13 [98.64]			
Ethambutol dihydrochloride	Etibi (500 mg) tablets	1.109	1.11 [1.09]	99.64 [98.28]	0.01 [0.011]	2.08 [4.55]	3.36
		2.218	2.20 [2.209]	99.19 [99.59]	0.02 [0.02]	0.78 [0.125]	1.56
		4.436	4.43 [4.429]	99.86 [99.84]	0.04 [0.05]	0.63 [0.05]	1.56
		6.654	6.55 [6.57]	98.44 [98.73]	0.06 [0.07]	4.40 [1.071]	1.36
			Average	99.28 [99.11]			

• Developed method

[••] Official method

Table 5: Accuracy and presecion for Isoniazid determinations in urine and. plasma samples* by method B.

Sample	Taken (mg ml^{-1})	Found (mg ml^{-1})•[••]	Recovery (%)•[••]	± S.D. (mg ml^{-1})•[••]	t- value•[••]	F-test
Urine	1.097	1.10 [1.11]	100.63 [101.19]	0.012 [0.02]	0.42 [0.63]	2.78
	2.194	2.21 [2.22]	100.59 [101.23]	0.018 [0.022]	3.05 [4.66]	1.49
	4.388	4.43 [4.42]	100.97 [100.73]	0.020 [0.035]	1.13 [0]	3.06
	6.582	6.65 [6.64]	100.95 [100.81]	0.025 [0.056]	2.95 [1.11]	5.07
		Average	100.79 [100.99]			
Plasma	1.097	1.12 [1.11]	102.10 [101.19]	0.015 [0.012]	3.00 [1.04]	1.56
	2.194	2.20 [2.2]	100.27 [100.27]	0.024 [0.03]	1.56 [1.67]	1.56
	4.388	4.41 [4.45]	100.50 [101.41]	0.034 [0.046]	2.21 [1.63]	1.83
	6.582	6.63 [6.68]	100.73 [101.49]	0.047 [0.051]	0.77 [3.43]	1.18
		Average	100.90 [101.09]			

• Developed Method

[••] Official Method

* Average of three determinations

Table 6: Accuracy and presecion for Ethambutol determinations in urine and.. plasma samples* by method

Bsample	Taken (mg ml^{-1})	Found (mg ml^{-1})•[••]	Recovery (%)•[••]	± S.D. (mg ml^{-1})•[••]	t- value•[••]	F-test
Urine	110.9	110.40 [111]	99.55 [100.09]	1.20 [0.9]	0.83 [0]	1.78
	221.8	220.70 [222.1]	99.50 [100.14]	1.80 [2.2]	0.28 [1.25]	1.49
	443.6	443.10 [442]	99.89 [99.64]	2.00 [3.5]	1.12 [0.71]	3.06
	665.4	664.50 [663.5]	99.86 [99.71]	2.50 [5.63]	1.05 [1.55]	5.07
		Average	99.70 [99.89]			
Plasma	110.9	111.00 [112]	100.09 [100.99]	1.20 [1.5]	0.00 [3.33]	1.56
	221.8	220.00 [220]	99.19 [99.19]	3.00 [2.4]	0.83 [0.52]	1.56
	443.6	445.00 [441]	100.32 [99.41]	4.60 [3.4]	1.09 [2.21]	1.83
	665.4	668.00 [663]	100.39 [99.64]	5.10 [4.7]	3.92 [1.36]	1.18
		Average	100.00 [99.81]			

• Developed method

[••] Official method

* Average of three determinations

method B which reflects high accuracy, in addition to the high precision indicated by very low values of relative standard deviations.

The performance of the proposed methods were assessed by calculation of t and f values compared with the official methods. Mean values obtained in the students showed the absence of systematic errors in each method.

For Biological Fluids (Urine and Plasma): Different serial dilutions of biological fluid were carried out and the previous general procedure was applied without adding the drug. For method A up to (1:1000) final dilution, the resulted absorbance becomes zero reflects high degree of interference. In (1:10,000) final dilution, the 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 Less than (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 B 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 5 and 6.

In method A for urine and plasma fluids average recoveries in the ranges 98.99%-100.61% and 97.57%-100.40% respectively. t-values of 0.02-6.70 and 0.44-5.83 for urine, plasma samples respectively. In method B for urine samples average recoveries in the ranges 100.27%-101.18% and 99.50%-101.44% respectively. t-values of 0.42-4.86 and 0.28-4.55 for Isoniazid and Ethambutol dihydrochloride and for plasma samples average

recoveries in the ranges 100.27%-103.01% and 99.19%-102.8% respectively. t-values of 0.77-3.89 and 0.00-3.92 for Isoniazid and Ethambutol dihydrochloride respectively. 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 and assisted by applying F-test which less than the theoretical one at 95% confidence level confirms high precision and accuracy.

Previous general procedure was applied successfully. For method B up to (1:1000) final dilution, the region of determination becomes loudly reflects high degree of interference. In (1:10,000)

CONCLUSION

The linearity, sensitivity and reproducibility were the best. Moreover these procedures are found very suitable for the routine analysis of Isoniazid and the other investigated drugs in some pharmaceutical preparations without any excipients interference. For biological fluids as urine and plasma the proposed procedures were applied to determine the studied drugs using the standard addition technique. The results obtained compared by the official U.S.P. HPLC [2] methods where the results demonstrate good accuracies and precisions.

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