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Spectrophotometric Determination of Isoniazid in Dosage forms by Derivatization

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Abstract

Isoniazid (INH) anti-tuberculosis drug was determined spectrophotometrically after derivatizing with 2-hydroxy-1,4-naphthoquinone (HNQ) at pH 3 in aqueous-methanolic solution. The reaction conditions were optimized and the derivative absorbed maximally at 365 nm .The Beer's law was obeyed in the range 5-25µg/ml. With coefficient of determination 0.9991.The method was applied for the determination of INH in pharmaceutical preparations with coefficient of variation 0.86-0.98.

Introduction

Isoniazid (INH) is commonly used for the treatment of tuberculosis as the first line as well as second line drug. It inhibits the growth of tubercle bacillus in vitro in concentration less than 1µg/ml. The INH gains access to all organs and all body fluids including cerebrospinal fluid, renders the drug of special value in treating tuberculosis meningitis and other extra pulmonary forms of disease. When used alone, it is at least equal to streptomycin in the therapy of tuberculosis.

The analysis of INH is based on titrimetry [1-3], spectrophotometry [4-7], spectrofluorometry [8-9], thin layer chromatography [10, 11], high performance liquid chromatography [12-16], gas chromatography [17-20] and electroanalytical techniques [21-23]. The spectrphotometric methods are simple and required sensitivity is achieved using a suitable derivatizing reagent.

For spectrophotometry a number of derivatizing reagents have been used including chloranil [24], sodium nitroprusside [25], neotetrazolium chloride, tetrazolium violet chloride [26], 2,3,5,-triphenyltetrazolium chloride [27], 2-nitroindan-1,3-dione [28], ethyl-8-quinolyloxylacetate [29], tetrazolium blue chloride [30], 2,6-dimethoxy-1,4-benzoquinone[6], 1-fluoro-2,4-dinitrobenzene [31], 4-chloro-5,7-dinitrobenzofuran [32], 6,7-dichloroquinoline-5,8-dione [33], 4-nitrobenzaldehyde [34], pyridoxal [34] and 4-dimethylaminobenzaldehyde [35]. The present work examines the use of 2-hydroxy-1,4-naphthoquinone (HNQ) as a derivatizing reagent for the spectrophotometric determination of Isoniazid.

Experimental

Materials and Reagents

All the chemicals and reagents used were of analytical or pharmaceutical grades. The double distilled water was used throughout the study. Pure INH was obtained from Nabi Qasim Pharmaceuticals Karachi, rifampicin was from Abbott Lab. Pak. Karachi and 2-hydroxy-1,4-naphthoquinone from E.Merck(Germany).

Buffer solutions between pH 1-10 at unit interval were prepared from hydrochloric acid (1M), potassium chloride (1M), acetic acid (1M), sodium acetate (1M), sodium bicarbonate (1M), sodium carbonate (saturated), ammonium chloride (1M) and ammonia solution (1M).

Spectrophotometric studies were carried out with double beam Hitachi 220 (Hitachi (Pvt.), Ltd, Tokyo, Japan) spectrophotometer with dual quartz cuvettes. pH measurements were made with Orion model 420A pH meter with glass electrode and combined reference electrode (Orion Research Inc. Boston, USA).

Analytical Procedure

The solution (0.5-2.5ml) containing INH 50-250µg was transferred to 10 ml volumetric flasks and was added HNQ (2ml, 0.05% in ethanol) followed by sodium acetate buffer pH 3 (1ml). The contents were heated on water bath at 70°C for 15 min. The solution was cooled at room temperature and volume was adjusted to mark with methanol. The absorbance was measured at 365nm against reagent blank, prepared in a similar way, omitting the addition of INH.

Analysis of Isoniazid in Pharmaceutical Preparations

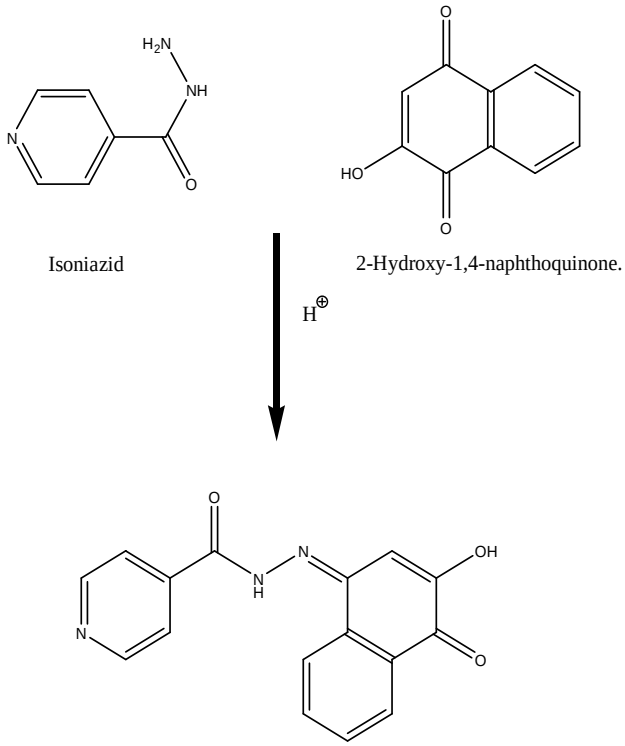
Twenty tablets each Isoniazid B.P (Feroz son's laboratories ,Standard pharmaceutical Ltd: Noshera, (Pak)) or Mayambutol INH (Lederle laboratories Cyanamid (Pak) Ltd: Karachi were thoroughly ground to fine powder and the amount 0.2118g and 0.6030 corresponding to 100 mg and 300 mg INH were transferred to separate beakers and dissolved in water. The solutions were filtered and the volumes were adjusted to 100ml. The solution 5ml from each was further diluted to 100ml and finally solution 2ml was transferred to 10ml volumetric flask. The above derivatization procedure was followed as described in A.

Determination of Isoniazid in the presence of Rifampicin

20 tablets each Rifinah 450 (Pacific Pharmaceutical Ltd. Lahore, Pak) or Rimactazid 450 (Ciba Giegy (Pak) Ltd. Karachi) were thoroughly ground to fine powder and the samples 0.16661g and 0.98115g respectively were dissolved in ethanol with 8-10ml portions, for five times on water bath in two separate flasks .The solutions were filtered and the final volumes were adjusted to 100ml. Again 1ml from each flask was further diluted to 10ml with ethanol. Finally 1-2ml from each flask were transferred to separating funnels and were added 2ml water,1ml buffer solution pH 3 and 3-4ml chloroform. The contents were mixed well and layers were allowed to separate. The organic layer was collected and the extraction was repeated with 2-3 ml of chloroform. The volumes of organic layers were adjusted to 10ml with chloroform and the absorbance was measured at 475 nm for the determination of rifampicin. The aqueous layer was transferred to separate volumetric flasks (10 ml) and the remaining procedure was followed as described above (A). The amounts of Isoniazid and rifampicin in tablets were calculated from the external calibration curve prepared from standard solutions of isoniazid and rifampicin (n=5) in a mixture following above procedure.

Results and Discussion

Isoniazid reacts with 2-hydroxy-1, 4-naphthoquinone (HNQ) in 1:1 molar ratio to form a derivative Isonicotinic acid(3-hydroxy-4-oxo-4H-naphthalene-1-ylidene)-hydrazide (INH-HNQ). (Fig.1) which absorbs at 365 nm with molar absorptivity 1.9×10^4 L $mole^{-1}$ cm^{-1} .The absorbance of the derivative after its formation was measured at different intervals of time and any change in absorbance was not observed up to 24 hrs. The effects of pH, reagent concentration and heating time and temperature on the derivatization reactions were examined. The pH effects within 1 to 10 at unit-interval was studied and it was observed that maximum absorbance was observed at pH 3 (Fig. 2) and sodium acetate buffer pH 3 covered pH range satisfactorily. The amount of derivatizing reagent added (0.05% in ethanol) was varied from 0.5 to 3.0 ml at an interval of 0.5 ml and addition of 2.0 ml was found to be optimal. The heating time and temperature were examined from 5 to 25 min at 70 °C at an interval of five min, and a similar response was observed after heating for 10min. Therefore heating time for 15 min was considered as optimum (Fig. 3). The effect of variation in the concentration on the absorbance was examined and the derivative obeyed the Beer's law within the concentration range 5-25 µg/ml INH with coefficient of determination (R^2) 0.9991.



Isoniazid

2-Hydroxy-1,4-naphthoquinone.

Isonicotinic acid(3-hydroxy-4-oxo-4H-naphthalene-1-ylidene)-hydrazide

Fig.1. Structural formula of the isoniazid derivative Isonicotinic acid (3-hydroxy-4-oxo-4H-naphthalene-1-ylidene)-hydrazide (INH-HNQ).

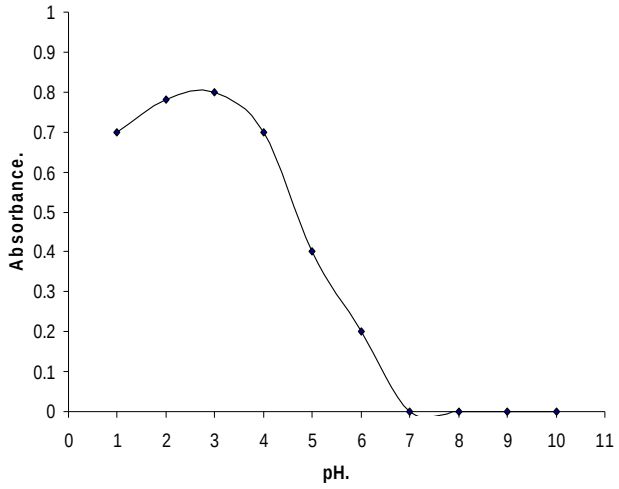


Fig.2. Effect of pH on derivatization of INH.

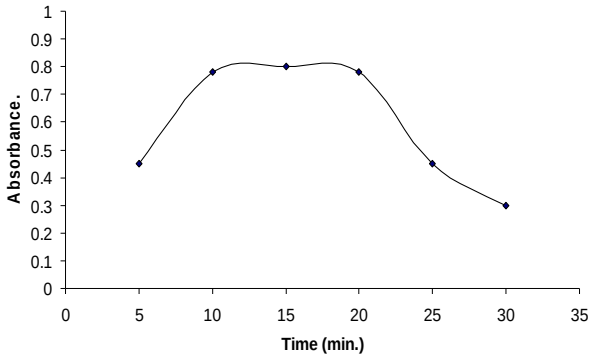


Fig. 3. Effect of heating on derivatization of INH.

Isoniazid is present in pharmaceutical preparations individually and in combination with ethambutol, rifampicin and pyrazinamide .The effect of their presence on the spectrophotometric determination of isoniazid was therefore examined. It was observed that ethambutol did not effect the determination when present twice the concentration of isoniazid. Rifampicin also absorbs at 365nm and effects the determination. However at pH 3 rifampicin from aqueous ethanolic solution transferred to chloroform to facilitate the determination of isoniazid in aqueous ethanolic phase. Rifampicin in the chloroform could be determined after measuring the absorbance at 475 nm. The effect of pyrazinamide was also examined, but some enhancement in the absorbance of isoniazid was observed when present in twice the concentration of isoniazid. However, it did not effect at the same concentration of Isoniazid (5µg/ml). The effect of the possible presence of additives such as methylparaben, propylparaben, gum acacia, magnesium stearate, lactose, glucose and starch in the pharmaceutical preparations were investigated at ten times the concentration of INH and none of these substances interfered, and change in absorbance as compared to standard was less than 5% (Table 1).

The method was applied for the determination of INH in pharmaceutical preparations. The results obtained were found in good agreement with the labeled values reported by the manufacturers. The coefficient of variation was obtained within 0.86-0.98 with relative deviation of 2.2-2.6% (Table 2).

Table 1. Effects of different interfering drugs / additives on the absorbance of 5µg/ml INH as derivative of HNQ

S.No	Compounds added	Absorbance
1	----------	0.69
2	Ethambutol 10µg/ml	0.68
3	Pyrazinamide 5µg/ml	0.72
4	Methylparaben 10µg/ml	0.68
5	Propylparaben 10µg/ml	0.62
6	Gum acacia 10µg/ml	0.63
7	Magnesium Stearate 10µg/ml	0.65
8	Lactose 10µg/ml	0.66
9	Glucose 10µg/ml	0.68
10	Starch 10µg/ml	0.68

The results obtained were also compared with a reported HPLC procedure [36] and an agreement of the results with HPLC procedure was obtained.

Conclusion

Simple Spectrophotometric method has been developed for the determination of INH using HNQ as derivatizing reagent with linear calibration range within 5-25µg/ml. The method was used for the determination of INH from pharmaceutical preparations. The additives present did not effect the determination.

Table 2. Analysis of Isoniazid and Rifampicin in pharmaceutical preparations

S.NO.	Name of Tablet.	Compounds Present.	Amount reported by the Manufacturer mg/tablet.	Amount found mg/tablet (C.V%).	%Relative deviation.	Amount found by HPLC method mg/tablet (C.V%)
1.	Isoniazid B.P.	Isoniazid	100	96.4 (2.86)	3.6	---------
2.	Mayambutol INH.	Isoniazid	100	99.8 (2.78)	0.2	--------
		Ethambutol	---------	---------	---------	--------
3.	Rifinah.450.	Isoniazid	300	286.30 (1.65)	4.56	288.5 (3.15)
		Rifampicin	450	430.1 (2.3)	4.42	430 (3.90)
4.	Rimactazid 450.	Isoniazid	300	287.31 (2.1)	4.23	289.9 (1.75)
		Rifampicin	450	432.2 (2.2)	3.95	328 (3.82)

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