"Spectrophotometric Determination of Isonicotinic Acid Hydrazide"

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Introduction

Isonicotinic acid hydrazide (isoniazid) is a specific drug for the treatment of tuberculosis. Many colorimetric and spectrophotometric methods [1-12] have been reported for the determination of isonicotinic acid hydrazide but most of the methods are not free from interferences [12] and cannot be used selectively for the determination of this compound.

During a systematic study of colour reactions, it was found that an acidic solution of vanillin gives beautiful yellow colortion with isonicotinic acid hydrazide which obeys Beer's law in the rang 20-160 ug/20 ml. The colour reaction has about 1 ugm/ml as the visual limit of identification and is specific for isonicotinic acid hydrazide when applied to pharmaceutical preparations. The present method is fairly simple, reproducible and economical.

Many pharmaceutical preparations have been evaluated by the present procedure. Allied compounds like aspirin, penicillin, streptomycin, ethambutol HCl and paracetamol do not interfere even when large amounts of these substances are present. Reference to the literature indicates that this method has not been reported previously.

Experimental

Apparatus

All absorbance measurements were made with double beam Beckman Spectrophotometer using 1-cm cells.

The pH meter was a Beckman combined electrode, Graduated pipettes accurate to ± 0.005 ml were used.

Reagents

All reagents used were of analytical grade or comparable purity.

Isonicotinic acid hydrazide (BDH) was recrystallized from 80% methanol (m.p. 171°C after recrystallization [9]). A 0.1% aquous solution was made which was further diluted to 0.002% solution.

O-vanillin, 10 mg/ml solution, was prepared in ethanol. A 7-day old solution kept at room temperature (30°C) gave the same results as a freshly prepared solution.

Procedure

Spot Test

To 1-2 ml of the test solution was added 1 ml of 2% solution of o-vanillin followed by 1 ml of glacial acetic acid. Appearance of yellow colour after about 10 minutes indicated the presence of isonicotinic acid hydrazide. Instead of glacial acetic acid, dilute hydrochloric or sulphuric acid can also be employed.

Calibration graph

A standard graph is prepared by taking different amounts of standard isonicotinic acid hydrazide. To 1.0 ml of a test solution containing 20 to 150

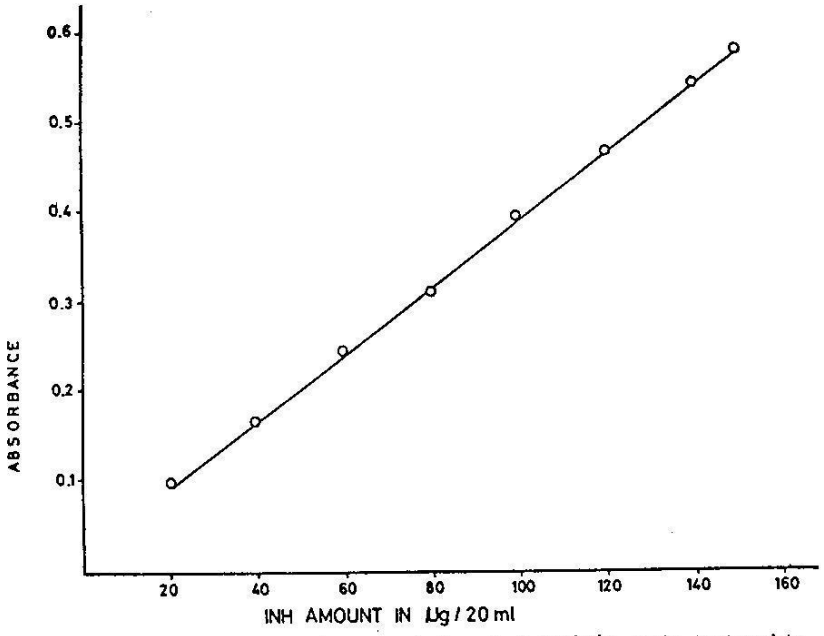

Fig.1: Typical calibration curve for ionicotinic acid hydrazide.

ug of isonicotinic acid hydrazide was added 1 ml of glacial acetic acid or the pH of the solution set to about 2 by dilute hydrochloric acid and add 1 ml of colour producing reagent, O-vanillin. The volume was made up to 20 ml with distilled water and shake. The contents were kept for 10 minutes at room temperature (30°C). The absorbance was measured at 358 nm, using 1 cm glass cells. The calibration graph is shown in Fig. 1.

Pharmaceutical Samples

Pharmaceutical tablet containing isonicotinic acid hydrazide (INH) was dissolved in distilled water and the volume made up to 1000 ml. Further dilution was made, if required, in order to bring the concentration of 1NH within the determination limits as mentioned above.

Results and Discussion

Effect of pH

The colour is stable at pH 1.8 to 2.4. The colour intensity decreases with the fall or increase in pH (Fig. 2).

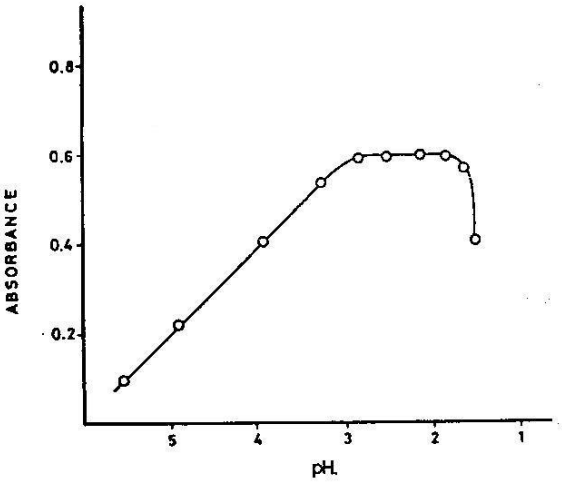

Fig. 2: Effect of pH on colour intensity.

Effect of time

the colour intensity attains its maximum in 10 minutes after mixing the solutions and then remains constant for more than six hours.

Effect of Temperature

The colour intensity remains constant in the range 20-75°C.

Table-I: Determination of Isonicotinic Acid Hydrazide (Isoniazid from Synthetic and Pharmaceutical Samples

Pharmaceutical/Synthetic sample	Amount of INH ug/20ml		Relative Standard Deviation %
	Present Actually	found	
Synthetic Mixture A	100	102	1.1
Synthetic Mixture B	150	153	2.0
Synthetic Mixture C	200	198	2.2
Synthetic Mixture D	250	250	0.0
*** P-Cetamol 20 mg + INH	100	102	1.2
*** Aspirin 10 mg + INH	100	100	0.0
*** Streptomycin 20 mg + INH	100	101	1.1
Isonexforte*	100[a]	98.5	1.5
Myambutol-INH**	200[b]	199.0	1.5
Myambutol**	Nil	Nil	-
Myambutol and Isonexforte	100[c]	99	0.9

* Pfizer Laboratories Limited, Karachi (Pakistan)

** Lederle Laboratories Division, Cyanamide (Pakistan) Ltd., Karachi.

*** The pharmaceutical preparations do not contain INH but these estimations have been made by the addition of INH in these preparations to find out their effect.

a Tablet containing 100 mg INH was dissolved to 1000 ml of distilled water and an aliquot of 1 ml taken for analysis.

b. Tablet containing 100 mg INH and 300 mg ethambutol was dissolved to 1000 ml of distilled water and an aliquot of 2 ml taken for analysis.

c. One tablet each of Myambutol and Isonexforte were dissolved to 1000 ml distilled water. An aliquot of 1 ml was taken for analysis.

Effect of concentration

For the complete development of colour the optimum amount of O-vanillin has been determined. It has been found that 10 mg of O-vanillin is required for 200 ug of isonicotinic acid hydrazide in a volume of 20 ml. Even the 10 fold excess dose not have any adverse effect on colour intensity.

Extinction Coefficient

The extinction coefficient of isonicotinic acid hydrazide and O-vanillin complex is of the order of 1.0×10^4 cm^2 mol^{-1}.

Order of Mixing of Reactants

The order of mixing of reagents has no effect on the development of colour.

Effect of other Aldehydes

The effect of other aldehydes and ketones on isonicotinic acid hydrazide has been studied (c.f. Table II).

The colour reaction of isonicotinic acid hydrazide with O-vanillin in acidic medium obeys Beer's law in the range 20-160 ug/ml of isonicotinic acid hydrazide (Fig.1).

The results for the determination of isonicotinic acid hydrazide are given in Table-I. Isonicotinic acid hydrazide has been determined routinely even when large amounts of paracetamol

Table-II Effect of Different Aldehydes and Ketones on Isonicotinic Acid Hydrazide and Acetic Acid.

Compound	Colour	Remarks
Veratraldehyde	No colour	
3,4 Dimethoxy Benzaldehyde*	No colour	
Crotonaldehyde*	No colour	
n-Heptaldehyde	No. colour	
Acetaldehyde	No colour	
Salicylaldehyde*	Light yellow	20 times less sensitive*
Propionaldehyde	No colour	
Formaldehyde	No colour	
Anisaldehyde	Light yellow	3 times less sensitive**
m-Nitrobenzaldehyde	No colour	
benzaldehyde	No colour	
o-Nitrobenzaldehyde	No colour	
Benzophenone	No colour	
Glucose	No colour	
Ascorbic acid	No colour	
o-vanillin	Yellow	Most sensitive

* Original compound itself is coloured

** Sensitivity standard comparison is vanillin

(200-fold) streptomycin (200-fold), ethambutol (500-fold) and aspirin (100-fold) are present (c.f. Table-I). Different pharmaceutical preparations containing isonicotinic acid hydrazide have been analytically evaluated by the present procedure (c.f. Table-I). The mode of the reaction appeared to be an acid catalysed reaction of carbonyl group with nitrogen to form hydrazone.

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