Spectrophotometic Determination of Isoniazid (INH) Using 2,4-dinitro-1-flourobenzene (DNFB) – Application to Tablet

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الخلاصة:

تم تطوير طريقة طيفية سهلة لتقدير كميات مايكرو غرامي ة من عقار الايزونيازايد في الاقراص الدوائية وتعتمد الطريقة على التفاعل بين الايزونزايد والكاشف (2,4–ثنائي نيترو – 1– فلورو بنزين) لتكوين صبغة ذات لون اصفر في الوسط القاعدي ذائبة في الماء ومستقرة بوج ود محلول منظم pH (10.3) . لقد وجد ان الناتج يمتلك طيفا امتصاصيا له أقصى امتصاص عند طول موجي 428 نانوميتر.امكن تطبيق قانون بيير ضمن مدى التراكز (0.05- 2.5) مايكرو غرام/مللتر في حين كانت الامتصاصية المولارية 2.7 x 10^4 لتر.مول$^{-1}$ سم$^{-1}$ والحساسية نسبةً الى معامل ساندل حد الكشف (0.0098) مايكرو غرا م/مل 0.005 مايكرو غرا م/سم^{٢} ومعدل نسبة الاسترجاع 99.99 % في حين كان الانحراف القياسي النسبي افضل من ±1.0 % كذلك وجد ان الناتج يتكون بنسبة 1:1 وان ثابت الاستقرار 5.9 x 10^3 لتر.مول$^{-1}$ وقد تم تطبيق الطريقة بنجاح على عدد من الم ستحضرات الصيدلانية الحاوية على الايزونزايد وتمت مقارنة الطريقة المقترحة مع الطريقة المعتمدة في دستور الادوية البريطاني.

Abstract

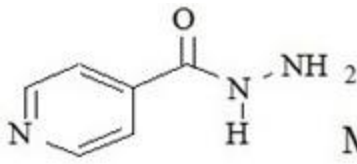
A rapid, simple, reproducible and sensitive spectrohpotometric method for assay of (INH) was investigated. The method is based on the reaction of INH with 2,4-dinitro-1-flourobenzene reagent to give a highly colored species with maximum at λ 428 nm in aqueous medium of pH 10.3. Beer's law was obeyed in the range of (0.05 – 2.5) µg/ml with a molar

absorptivity 2.7 x 10^4 l.mol l^{-1} cm^{-1} .The sandell index was 0.005 µg.cm^{-2} and the limit of detection was 0.00984 µg/ml. The accuracy (average recovery%) was 99.99% and the relative standard deviation (RSD) was better than ±1.0%. Also it was found that the product formed was in a ratio of 1:1 with a stability constant of 5.9 x 10^3 l.mol^{-1}. The method was applied successfully to assay INH in pharmaceutical formulations and agreed well with its certified value and the British pharmacopoeia method.

Keywords: Spectrophotometric; Isoniazid; DNFB.

Introduction

Pyridine - 4 - carboxylic acid hydrazide [I] commercially known as isoniazid is an important drug compound for the chemotherapy of tuberculosis and is widely used together with rifampicin and streptomycin[(1)]. This prompted many investigators to devise methods for the rapid determination of isoniazid in its pure form as well as pharmaceutical preparations.



[I]

$C_6H_7N_3O$

M .W t = 137.1 g/mol

m .p = 170 - 174 ^{0}C

Many analytical techniques have been proposed for the determination of isoniazid including fluorescence[(2,3)], electroanalytical[(4)], flow-injection chemiluminescene methods[(5)], titrations[(6,7)], atomic absorption spectrometry[(8,9)] chromatography[(10)], capillaryelectrophoresis[(11,12)] and H^1-NMR spectroscopy[(13)]. The above mentioned techniques are sensitive but expensive, Specetrophotometry[(14,15)] is still the technique of choice even today due to inherent simplicity.

In the literature many spectrophotometric procedure have been applied for the determination of isoniazid using different reagents including 6-meythyl -2-pyridine carboxaldehyde as a derivatizing reagent[(16)], 1,2,4-aminonaphtholsulphonic acid[(17)], 6,7-dichloroquinoline-5,8-dione[(18)], 4,4-sulphonyldianiline as a coupling agent[(19)], and using molybdenum(VI) in acidic medium as an oxidizing agent[(20)] in addition to other spectrophotometric methods[(21,22,23)].

The British pharmacopoeia[24] atitrimetric reported method using potassium bromate solution as atitrant in acidic medium for determination of isoniazid as a pure powder and in tablets and requires about 0.25 g of the drug. However 2,4-dinitro-1-flourobenzene (DNFB), so called Snager's reagent, has been used as achromogenic reagent for the spectrophotometric determination of amino acids primary and secondary amines[25-29], amino acid nitrogen in plasma and urine[30,31], isoniazid[32], various amino glycoside, antibiotics (gentamycin, torbomycin, amikacin)[33], phenols[34] and the enzyme amidase[35]. It has also been used in high-performance liquid chromatography for the determination of amines and aminoglycocides and in thin layer chromatography[36-38]. However these methods lack selectivity.

In this paper the employment of (DNFB) as achromogenic reagent for simple, sensitive, selective, rapid, accurate, precise and inexpensive method for determination of isoniazid in pure powder and pharmaceutical preparation is described.

Materials and Methods

Apparatus and reagents

Shimadzu (UV-210) double beam spectrophotometer with 1.0 cm silica cells was used to measure the absorbance and graduated pipettes were employed. Analytical grade chemicals and distilled water were used. Isoniazid (State Company for Drug Industries and Medical Appliances, Sammara-Iraq) standard solution(100 µg/ml) was prepared in distilled water to get a stock solution, which was diluted further as required, while $1x10^{-2}$M of DNFB (Sigma Co.) and buffer solution of pH 10.3[39] buffer solution was prepared from (sodium hydroxide and borate).

Reagents

DNFB (2,4-dinitro-1-flourobenzene) (1×10^{-2} M) solution was prepared daily by dissolving accurately 0.186 g of DNFB in about 5 ml ethanol then transferred to 100 ml volumetric flask and diluted to the mark by distilled water (the prepared solution is stable for several days if stored in a cool and dark place).

Buffer solution (pH 10.3)

The buffer was prepared by transferring 50 ml of 0.025 M solution of sodium tetraborate and 21.3 ml of 0.1 M solution of sodium hydroxide into 100 ml volumetric flask then diluted to the mark with distilled water.

General Procedure

Aliquots and calibration graph containing 0.05 to 2.5 ml (25 µg/ml) of INH were transferred into a series of 25- ml standard flasks, followed by addintion of 2 ml of 1 x 10^{-2} M DNFB and 1.5 ml of pH 10.3 and diluted to the mark with distilled water. After 30 minute standing time at room temperature the absorbance of the yellow-colored product formed was measured at 428 nm against a reagent blank. A calibration curve was then constricted (Fig.1). The color reaction obeys Beer's Low from 0.05 to 2.5 µg/ml of INH. The molar absorptivity, Sandell index, and limit of detection were calculated and found to be ,2.7 x 10^{7}, 0.005 and 0.0098, respectively.

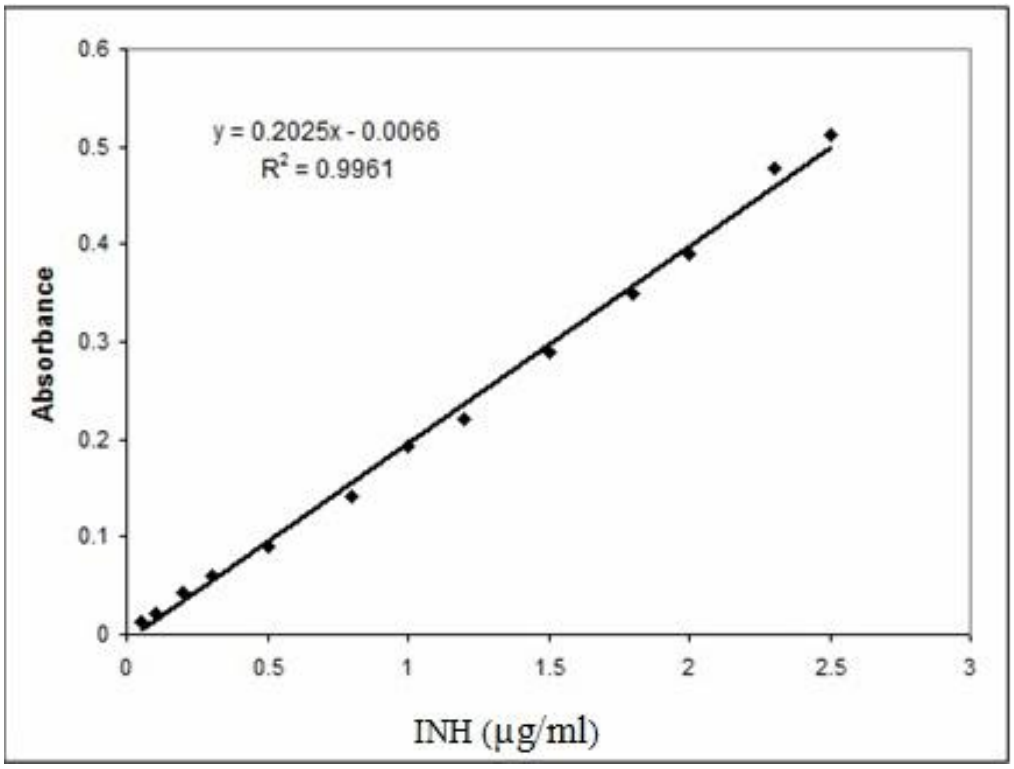


Figure 1: Calibration curve of INH using DNFB as a reagent

Procedure for determination of isoniazid (INH) in pharmaceutical preparations

Ten tablets (each tablet containing 10 mg isoniazid) were accurately weighed and pulverized. A portion of the fine and homogenized powder weighed dissolved and transferred to 100-ml volumetric flask and diluted to the mark with distilled water to get 100 µg/ml solution. From this solution 25 ppm was prepared and used as a test solution. Aliquots of the tablets solution were taken and the procedure as described above was followed.

Table 1: Assay of INH drug in some pharmaceutical formulations by the proposed method.

Procedure applied	Pharmaceutical Preparation	Drug amount taken (µg/ml)	Recovery % n=5	Drug constant found	Average recovery %	Certified Value (mg)
Proposed method	Tablet IPI, Iraq	0.5	99.99	0.499	99.98	10 mg
		1	99.97	0.997		
		2	100.0	2		
	Tablet Macleods, India	0.5	99.97	0.492	100.003	
		1	100.03	1.003		
		2	100.01	1.981		

Results and Discussion

Absorption spectrum of the colored complex

Isoniazid reacts with DNFB in a basic medium (pH 10.3) at room temperature to give a yellow colored complex, the absorption spectrum of which shows a maximum at 428 nm in [Fig(2)].

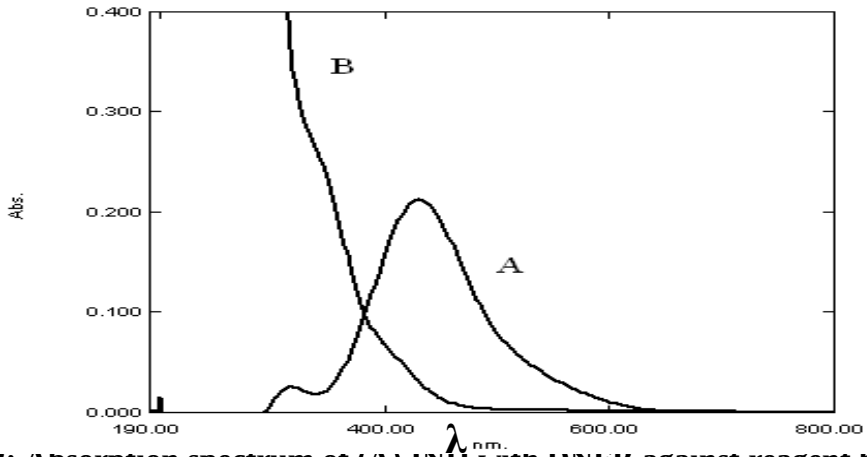


Figure 2: Absorption spectrum of (A) INH with DNFB against reagent blank, and (B) reagent blank againts distilled water.

Optimization of variables

Effect of pH and buffer solution

The effect of pH on the absorption of the prodeuct was studied using different pHs of NaOH ranged from 6-11. It was found that the product formed with maximum absorption when the final pH of the solution was 10.3 (Table 2). Therefore different buffers with pH 10.3 where prepared to

examin the sensitivity. It was found that borat buffer solution with pH 10.3 gave maximum absorpance (Table 3) which is used in subsequent experiments.

Table 2: Effect of NaOH amount on the absorbance

NaOH 1×10^{-2} M ml	0	0.5	1	1.5	2	2.5	3	3.5
Absorbance	0.002	0.096	0.275	0.36	0.449	0.371	0.33	0.22
Final pH of the reaction mixture	6.3	7.3	9.6	10	10.3	10.6	10.77	11.09

Table 3: Effect of buffers on the absorbance

Buffer solution	With out	B1	B2	B3	B4	B5	B6
Absorbance	0.002	0.285	0.385	0.47	0.50	0.54	0.49
Final pH of the reaction mixture	6.3	9.72	9.95	9.66	10	10.3	10.6

B1= $NH_3 – NH_4Cl$
B2= $Na_2CO_3 – NaHCO_3$ } (pH 10.3)
B3= $NaHCO_3 – NaOH$
B4= $Na_2B4O_7 – NaOH$ (pH 10)
B5= $Na_2B_4O_7 – NaOH$ (pH 10.3)
B6= $Na_2B_4O_7 – NaOH$ (pH 10.6)
-It was found that 1.5 ml of buffer B5(pH 10.3)solution has been selected.

Effect of temperature and reaction time

Full colored product was develop rapidly after the sequence addition of the reagents and the maximum absorbance was attained after 30 minute at room temperature. The color was stable for a period of more than 60 minute after which it begun to fade.

Effect of the amount of DNFB reagent

The influence of DNFB concentration on the color intensity was studied by measuring the absorbance at the specified wavelength in the proposed procedure for solutions containing the same drug amount and

optimum amount of the buffer but varying amount of DNFB. A volume of 2.5 ml was found to be sufficient for full color development.

Effect of order of addition

To obtain optimum results, the order of addition of reagents was checked and was found to be immaterial.

Accuracy and Precision

The accuracy and precision of the calibration curve was established by measuring the content of INH in pure form at three different concentration levels (Table 4). The values of the relative standard deviation and mean percent recovery obtained by the proposed method can be considered to be very satisfactory.

Table 4: Precision and accuracy of the proposed method.

Amount Added µg/ml	Recovery % n=5	Average Recovery %	*RSD
0.3	100.004		2.04
1	99.99	99.99	0.682
2.5	99.99		0.289

*Average of five determinations.

Nature of the colored product

The molar ratio of the product formed between the drug and the DNFB reagent was established using continuous variations (Job's) method[40]. The result indicated that the product was found in the ratio of 1:1(INH : DNFB) [Fig (3)].

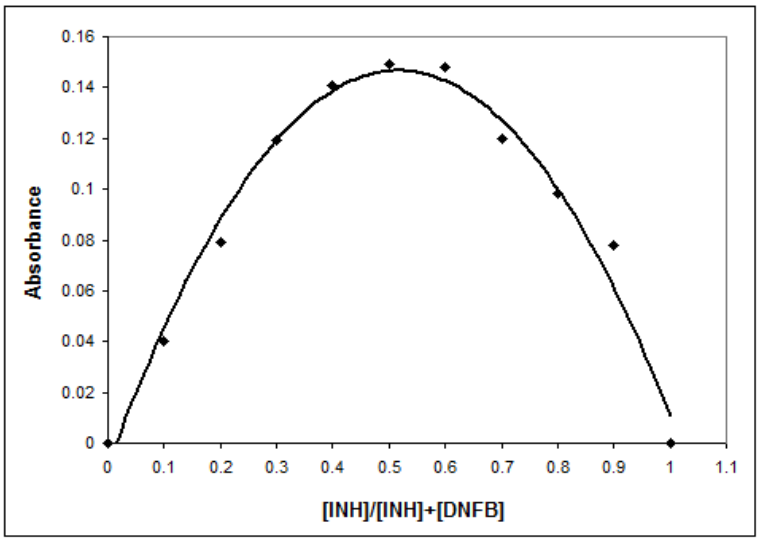
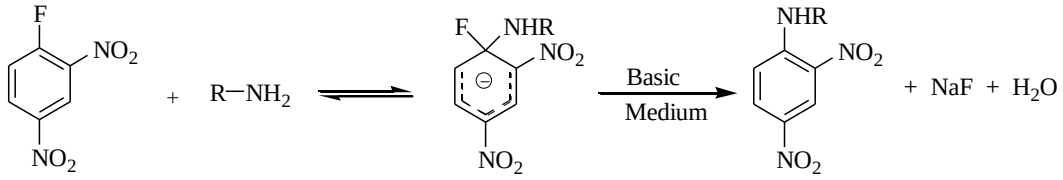


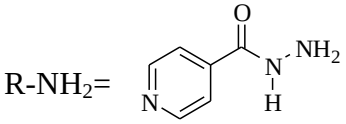
Figure 3: Job's plot of INH drug and DNFB interaction

Reaction Mechanism

The reaction of DNFB with drug that own a free primary amine group results in the formation of colored product [41]. This reaction was first introduced by Sanger[42] as means for determination of (DNA) sequence. Based on the job's method on the continuous variation, it was found that INH interacted with DNFB in ratio of 1:1 this result indicates that the reaction between the drug and the reagent used takes place only one site which was the more sterically free terminal amino group, the reaction is typical nucleophilic substitution and proceed thought an intermediate product as cited in scheme 1.



DNFB Intermediate Deep yellow product



Isoniazid

Scheme1: Probable reaction mechanism of INH with DNFB reagent.

Interferences

The extent of interference by some excipients which often accompany pharamaceutical preparations was determined by measuring the absorbance of solutions containing (25 µg) of INH and various amounts of diverse species in a final volume of 25 ml it was found that the studied excipients do not interfere even when present in large excess. An error 0f ±3% in the absorbance readings was considered tolerable. Typical results are given in (table 5).

Table 5: Effect of excipients for assay of 25 µg isoniazid

Excipients	Amount added µg	Relative Error E%	Recovery %
Glucose	1000 µg	-1.01	99.93
Lactose	=	-2.02	98.02
Dextrose	=	+1.01	100.01
Gum cacia	=	+3.03	99.96
Starch	=	+1.51	100.01
Nicotineamide	=	+0.505	101.55
Pyridoxine tab.(B6)	=	-1.02	99.99
Striptomycine sulphate	=	+2.03	100.02
Sodium chloride	=	+0.207	108.04

Application

The proposed method was successfully applied to determine INH tablet pharmaceutical preparation. The obtain result were compared statically[43,44] by a student's t-test for accuracy with official method at the 95% confidence label with 5 degrees of freedom as cited (Table 6). The result showed that the experimental t-test was less than the theoretical value (2.776) indicating that there was no significant difference between the proposed method and the official method.

Table 6: Assay of INH Drug in some Pharmaceutical formulations by the proposed methods and official method.

Procedure applied	Pharmaceutical preparation	Drug amount taken µg/ml	Recovery % n=5	Drug amount found	Average recovery %	Certified value mg	t-experimental (t-test)
Proposed method	Tablet (IPI, Iraq)	0.5	99.99	0.499	99.98	10 mg	t-experimental (t-test)
		1	99.97	0.997			
		2	100.0	2			
	Tablet Macleodes, India	0.5	99.97	0.492	100.003		
		1	100.03	1.003			
		2	100.01	1.981			
Official method		0.5	97.26	0.478	99.93		0.615
		1	101.75	1.020			0.560
		2	100.79	1.996			0.858

Comparison of methods

The present proposed method has been compared with other spectrophotometric methods as shown in (Table 7):

Table 7: Summary of optical characteristics and statistical data of the proposed method compared with the other method

Analytical Parameters	Diazotised reagents				
	D-fast red AL salt method [45]	**D-dapsone method [46]**	**D-PNA method [47]**	**D-SA method[48]**	**INH-DNFB proposed method**
Medium of reaction	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
λ max (nm)	510	440	525	410	428
Temperature (C°)	20 ± 3	20 – 30	room temperature	room temperature	room temperature
Development Time (min)	10	- - - - -	- - - - -	10	30
Stability Period (min)	20	60	60	50	60
Molar absorptivity $\times 10^{-4}$ ($l\ mol^{-1}\ cm^{-1}$)	0.85	0.57	2.13	0.59	2.7×10^4
Beer's Law range (ppm)	2 – 15	0.5 – 20	0.2 – 8	0.2 – 24	0.05 – 2.5
Average recovery (%)	- - - - -	- - - - -	- - - - -	99.47	99.99
RSD (%)	1.43	0.35	1.43	0.95	1.00
Stability constant (K)	- - - - -	- - - - -	$0.52 \times 10^5 M^{-1}$	$0.90 \times 10^9 M^{-2}$	5.9×10^3
Correlation coefficient	0.9993	0.9961	0.9998	0.9995	0.9961
Toxicity of reagent	Irritating[a]	Harmful[b]	Highly toxic[c]	Irritating[d]	Harmful
Analytical application	Phamaceutral preparation	Phamaceutral preparation	Phamaceutral preparation	Tablet	Tablets

Conclusion

An spectrophotometric method for the determination of INH was developed. The method is simple and sensitive. The statistical analysis a good agreement with those of the official British pharmacopoeia method. The color reaction is selective for INH. The method was successfully applied to the micro determination of INH either in a pure on in pharmaceutical preparations.

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