

Spectrophotometric Determination of Isoniazid in Pure Form and Pharmaceutical Preparation

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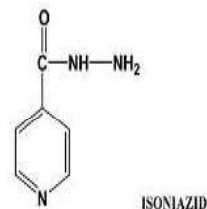
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Abstract: Simple spectrophotometric methods are described for the assay of isoniazid (INH) based on charge-transfer and ion-pair complexation reactions. The first method (A) is based on the reaction of drug with chloranilic acid (CA); tetracyanoethylene (TCNE) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in acetonitrile as a solvent. The maximum absorbance was measured after 15 min at 30 °C, at 500, 480 and 580 nm as respectively. The second method (B) is concerned with the reaction of investigated drug with thymol blue (TB); bromo-phenol blue (BPB) and bromo-cresol green (BCG). The ion-pair complexes formed show absorption spectra with maximum at 390, 410 and 320 nm as respectively. The methods are rapid, simple and do not require any separation step. Beer's law validation, accuracy, precision, limits of detection, limits of quantification and other aspects of analytical merit are presented in text. The proposed methods were applied for the determination of the analyses in pure form and pharmaceutical preparation. The results were in good agreement with those obtained by the official and reported methods.

Key words: Spectrophotometry • Isoniazid(INH) • Charge-transfer and Ion-pair complexation reaction

INTRODUCTION

Isoniazid (INH) pyridine-4-carboxylic acid hydrazide (Scheme 1) is a bacteriostatic drug and is now widely used together with other antituberculostatic agents for chemotherapy of tuberculosis. It also seems to be active for extrapulmonary illnesses such as meningitis and genito-urinary infections [1]. The determination of Isoniazid in pharmaceutical preparations and biological fluids has appeared especially attractive. There are various analytical procedures for the assay of INH, the most important being titrimetry [2,3], visible spectrophotometry [4-7], polarography [8], coulometry [9], high performance liquid chromatography [10,11], fluorimetry [12,13] and chemiluminescence [14,15] methods. These methods often suffer from a variety of disadvantages, such as use of sophisticated and time consuming techniques, require expensive instruments and are not available at most quality control laboratories. The official methods involve bromination and titration with standard nitrite solution [16,17]. The above mentioned π -acceptors have many applications for the analysis of different electron-donor compounds [18-23]. The present work studies the complexation reactions of INH with (CA); (TCNE); (DDQ); (TB); (BPB) and (BCG) in acetonitrile as organic solvent. The methods were applied



Scheme 1: Structure formula of isoniazid(INH)

for the assay of the cited drug in pure form and in pharmaceutical preparation. The results obtained are favorably comparable with those of the official or reported methods.

EXPERIMENTAL

Apparatus: A Shimadzu recording UV-240 spectrophotometer (Japanes make)with 1 cm-cell was used.

Materials: Authentic samples of Isonicotinic hydrazide (isoniazid) powder supplied from Fluka company, Germany.

Market samples of isocid 50 mg tablets, isocid fort 200 mg tablets produced by cid Company, Giza, Egypt.

Table 1: Analytical parameters for the complexation of isoniazid

Parameters	CA	TCNE	DDQ	TB	BPB	BPG
λ_{max} (nm)	500	480	580	390	410	320
Reagent (ml)	4	4	4	2	2	2
time (min)	15-60	15-60	15-60	0-60	0-60	0-60
Beer's law limit ($\mu\text{g ml}^{-1}$)	1.371-8.226	6.855-34.275	10.968-21.936	6.855-41.13	1.371-6.855	1.371-8.226
Molar absorptivity	0.0311	0.1178	0.2064	0.0574	0.1055	0.0863
Sandell sensitivity	0.276	1.0423×10^3	4.033×10^3	5.2×10^3	4.72×10^3	38.627
Standard deviation	0.497	0.13	0.0339	0.026	0.029	0.0355
Specific absorptivity	4.5×10^{-6}	0.017×10^{-3}	0.075×10^{-3}	8.4×10^{-6}	0.077×10^{-3}	0.063×10^{-3}
Regression equation			$Y = ax + b$			
Slope (a)	0.0045	0.017	0.075	0.0084	0.077	0.063
Intercept (b)	0.0286	0.002	-0.2	0.0849	0.660	0.183
Correlation coefficient (r)	0.997	0.995	0.93	0.995	0.989	0.999
R.S.D. (%)	0.025	0.913	0.237	0.885	1.096	1.29
Range of error	1.33	0.3529	0.08	0.07	0.0779	0.095

Preparation of Standard Isoniazid Solution: The stock solution of isoniazid INH ($1 \times 10^{-3}\text{M}$) was prepared by dissolving 0.0137gm of INH in acetonitrile and diluting to the mark in a 100ml measuring flask by the same solvent.

Reagents: All the reagents and acetonitrile as a solvent used were of analytical grades.

Preparation of solutions CA, TCNE, DDQ, TB, BPB and BCG ($1 \times 10^{-3}\text{M}$) were prepared in acetonitrile.

Preparation of (3%) Na acetate, bidistilled water was used through the study. All solutions were freshly prepared.

Procedures

Charge-transfer Method (A): An accurate volume 0.5-2.0 ml of ($1 \times 10^{-3}\text{M}$) of the standard solution of isoniazid (INH) was transferred into 10 ml measuring flask, mixed with 4 ml ($1 \times 10^{-3}\text{M}$) of CA, TCNE, and DDQ, diluted to 10ml with acetonitrile (in case of DDQ add 1ml 3% Na acetate). After 15 min at 30°C , the max. absorbance was measured at the specified wave length 500, 480 and 580 nm as respectively, against a reagent blank (Table 1).

Ion-pair Method (B): an accurate volume of 0.1-0.6 ml ($1 \times 10^{-3}\text{M}$) of standard solution of isoniazid was transported into 10ml measuring flask, mixed with 2 ml of ($1 \times 10^{-3}\text{M}$) BPB and BCG then diluted to 10ml with acetonitrile. In case of TB the accurate volume of standard solution of isoniazid 0.5-2.0 ml ($1 \times 10^{-3}\text{M}$) and add 4ml of ($1 \times 10^{-3}\text{M}$) of TB. The max. absorbance was measured directly at 390, 410 and 320 nm, respectively against a reagent blank (Table 1).

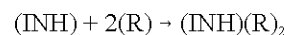
Application to Pharmaceutical Preparations: Twenty tablets were weighted and ground to finely divided powder. An accurate weight of the powder contain 6.8mg of isoniazid was dissolved in acetonitrile, the solution was

filtered and the clear solution was diluted to 100ml with acetonitrile, so that to produce ($1 \times 10^{-3}\text{M}$) final concentration. A 1-ml aliquot of this solution was used for the general assay procedures.

RESULTS AND DISCUSSION

Charge-transfer Method (A): Isoniazid (INH) was found to yield intense colours with the studied π -acceptors in aqueous-acetonitrile solutions, most probably due to the charge-transfer complexation between INH as n-donor and the π -acceptors with the subsequent formation of the highly coloured complexes (Fig. 1, 2, 3) shows the absorption spectra of the coloured products from INH. Absorbance measurements were taken at 500nm for CA, 560nm for TCNE and at 480nm for DDQ as INH products exhibited higher absorption intensities at these wave lengths.

Application of Job's continuous variation method [24] indicates a molar ratio of INH to acceptor of 1:2 (Fig. 4), shows the results obtained for INH with CA and TCNE, according to the following equation (1):



R: is the reagent(CA, TCNE, DDQ)

Ion-pair Method (B): The absorption spectra of the ion-pair complex formed between isoniazid and each of TB, BPB and BCG were measured immediately at 390, 410, and 320 nm respectively against a blank reagent and shown in (Fig. 5, 6, 7)

TB, BPB, and BCG were chosen to give the most stable and sensitive acid-dye procedure in the presence of the degradation products. The reaction stoichiometry of the associated ion-pair was found to be 1:1 using the three acid dyes, according to the following equation (2):

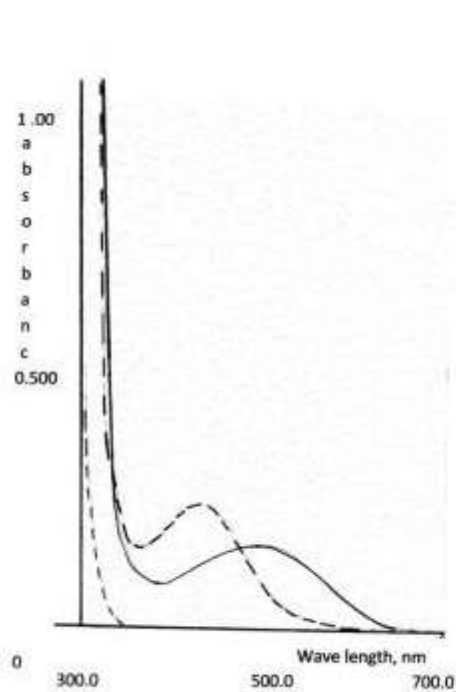


Fig.1 Absorption spectra of ..INH, -CA and -INH with CA at λ_{max} 500nm

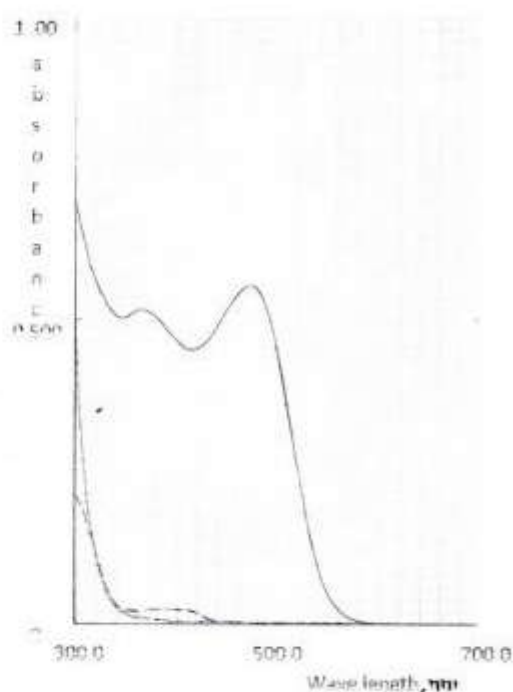


Fig.2 Absorption spectra of ..INH, -TCNE and -INH with TCNE at λ_{max} 480nm

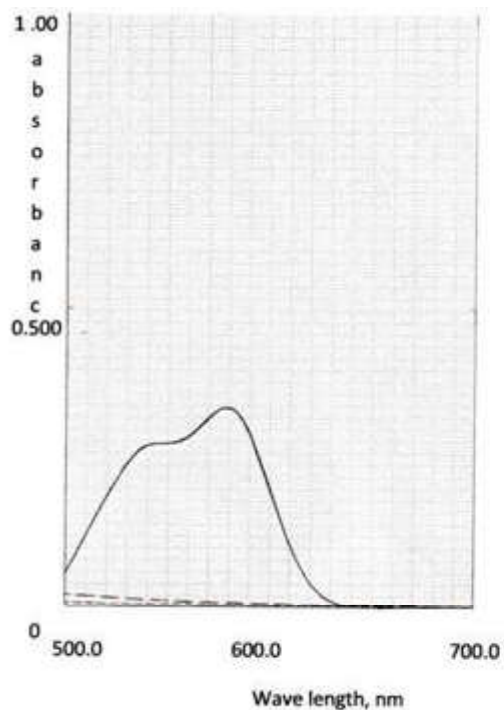


Fig.3 Absorption spectra of ..INH, -DDQ and -INH with DDQ at λ_{max} 580nm

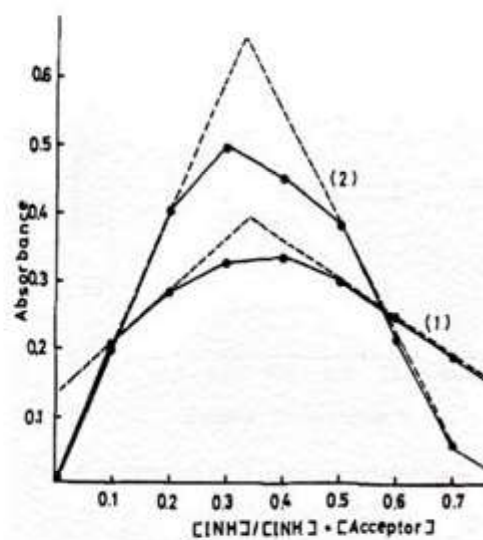


Fig.4 Continuous variation plot of INH and CA (1) and TCNE (2) Molar concentration for each ($1 \times 10^{-3}M$).

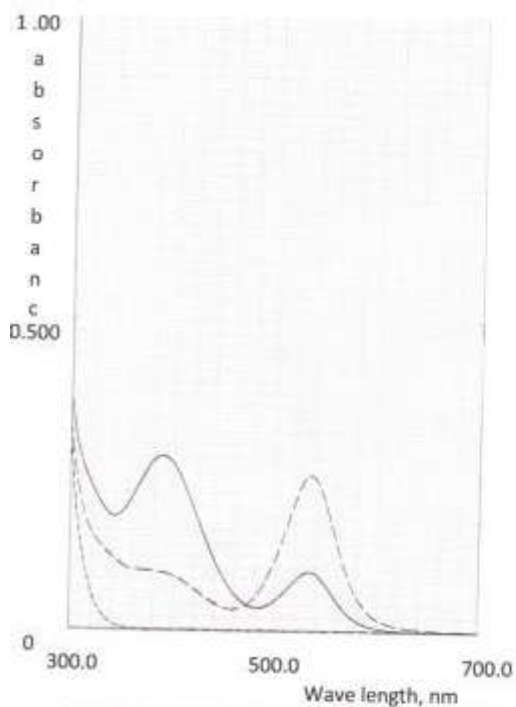


Fig.5 Absorption spectra of .. INH, -TB and -INH with TB at λ_{\max} 390nm

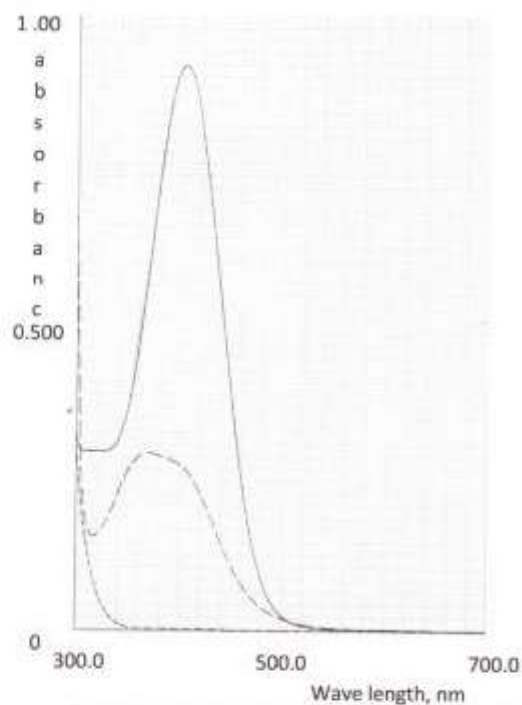


Fig.6 Absorption spectra of .. INH, -BPB and -INH with BPB at λ_{\max} 410nm

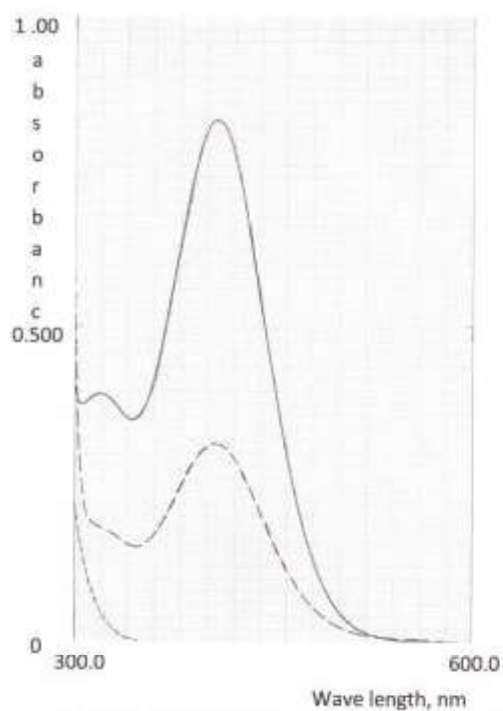


Fig.7 Absorption spectra of .. INH, -BCG and -INH with BCG at λ_{\max} 320nm

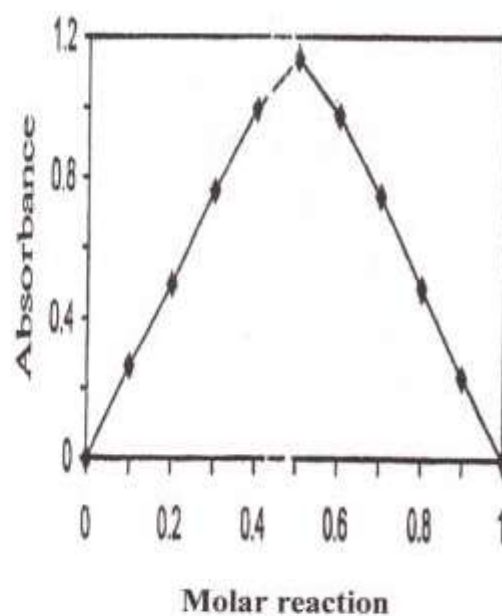


Fig.8 Continuous variation plot of INH-BCG complex ($1 \times 10^{-1} \text{M}$).

CONCLUSIONS

This paper described the two methods (charge-transfer and ion-pair) for the determination of INH in pure forms and pharmaceutical formulations. In terms of simplicity and accuracy, the proposed methods could be considered superior to the methods reported previously. These methods are suitable for the routine analysis of common bacteriostatic drug (isoniazid) in pharmaceutical preparations without interferences of any ingredients.

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