Spectrophotometric Determination of Isoniazid in Presence of Its Hydrazones

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Abstract □ A spectrophotometric determination of isoniazid in the presence of its hydrazones was developed. The method involves the reaction between isoniazid and 2,3-dichloro-1,4-naphthoquinone in the presence of ammonia in an ethanolic medium. The colored product has an absorbance maximum at 640 nm. The Lambert–Beer law is obeyed in the 1–14-μg/ml range. The proposed method was applied to the analysis of isoniazid tablets. In commercial tablets, hydrazone formation due to the reaction between isoniazid and lactose was detected by TLC. The analysis of lactose-containing isoniazid tablets showed 10–22% lower recovery than that obtained by the official method. Hydrazone formation in tablets probably interferes with isoniazid bioavailability.

Keyphrases □ Isoniazid—spectrophotometric analysis in presence of its hydrazones in prepared mixtures and commercial tablets □ Spectrophotometry—analysis, isoniazid in presence of its hydrazones in prepared mixtures and commercial tablets □ Tuberculostatic antibacterials—isoniazid, spectrophotometric analysis in presence of its hydrazones in prepared mixtures and commercial tablets

The analysis of isoniazid in dosage forms has been the subject of extensive study. Bromometric (1), nonaqueous titrimetric (2), and spectrophotometric (3) methods have been proposed. Isoniazid reacts with carbonyl compounds to form hydrazones (4). Interaction of isoniazid with lactose was investigated in the solid state using diffuse reflectance spectrophotometry (5). According to this study, the reaction does not proceed to any appreciable degree under ambient conditions. However, the interaction can become significant in the presence of high humidity and at elevated temperatures.

BACKGROUND

The presence of glucose and lactose isonicotinoyl hydrazones has been detected in various dosage forms, *e.g.*, syrups and tablets (6, 7). The interaction of glucose and lactose with isoniazid probably interferes with the bioavailability of isoniazid from its dosage forms. Absorption of lactose isonicotinoyl hydrazone was almost negligible in animals (7, 8). Surprisingly, as much as 60–70% of isoniazid was converted to glucose hydrazone within 24 hr in syrups. Pharmacokinetic study in humans revealed that the bound isoniazid is neither absorbed nor converted to the free isoniazid in the first 6 hr.

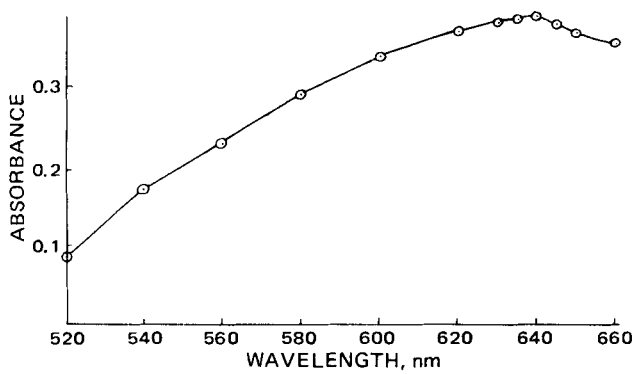

Figure 1—*Visible spectrum of the colored product obtained on reacting isoniazid with 2,3-dichloro-1,4-naphthoquinone.*

Recently, iodometric, nitrite, and bromometric methods, official in USP and BP, have been compared with the nonaqueous method (9). All official methods, as well as the nonaqueous titrimetric procedure, fail to make any distinction between isoniazid and its hydrazones. Therefore, it was of interest to develop a specific method for isoniazid in the presence of its hydrazones and to study the extent of hydrazone formation, if any, in tablets containing lactose as an adjuvant.

The proposed method is based on the reaction between isoniazid and 2,3-dichloro-1,4-naphthoquinone in ethanol. Reaction conditions such as the concentration of reagent and ammonia, time, and temperature have been standardized.

EXPERIMENTAL

Apparatus—All spectral measurements were carried out with a spectrophotometer[1] having four matched 10-ml cells of 1-cm light path.

Materials and Reagents—Ethanol BP, silica gel G[2], and isoniazid BP were employed. All other reagents were analytical grade.

2,3-Dichloro-1,4-naphthoquinone (10), glucose isonicotinoyl hydrazone (11), and lactose isonicotinoyl hydrazone (12) were synthesized as described previously.

Preparation of Ethanolic Ammonia (10% w/v)—Dry ammonia gas was passed through absolute ethanol at −5° until its weight had increased ~20%. The resulting solution was diluted with absolute ethanol to obtain a 10% (w/v) ammonia solution. The solution was stored at 5° in a refrigerator.

2,3-Dichloro-1,4-naphthoquinone Solution—This solution contained 0.034% (w/v) 2,3-dichloro-1,4-naphthoquinone in ethanol.

Standard Solutions—The following were used: 0.01% (w/v) isoniazid in ethanol (freshly prepared), 0.035% (w/v) lactose isonicotinoyl hydrazone in ethanol, and 0.022% (w/v) glucose isonicotinoyl hydrazone in ethanol.

Detection of Lactose Isonicotinoyl Hydrazone, Glucose Isonicotinoyl Hydrazone, Lactose, and Glucose—TLC, as described earlier (5, 13), was used to detect these compounds.

Determination of Wavelength of Maximum Absorbance—To the mixture of standard solution of isoniazid (2 ml) and ethanolic ammonia (6 ml), placed in a small flask and cooled in an ice bath, was added 2,3-dichloro-1,4-naphthoquinone solution (10 ml). The reaction mixture was kept in an ice bath for 25 min. The solution then was transferred quantitatively to a 25-ml volumetric flask with the aid of ice-cold ethanol, and the final volume was adjusted with cold ethanol. The reaction flask was placed in an ice bath. After 5 min, the absorbance was measured at 10-nm intervals from 520 to 660 nm against a blank. The blank contained 2,3-dichloro-1,4-naphthoquinone solution (10 ml) and ethanolic ammonia (6 ml) diluted to 25 ml with ethanol (Fig. 1).

Analysis of Isoniazid in Presence of Its Hydrazones in Synthetic Mixture—Aliquots of ethanolic isoniazid solution and its hydrazone solution were mixed with ammonia (6 ml) and cooled in an ice bath (Table I). 2,3-Dichloro-1,4-naphthoquinone solution (10 ml) was added to the mixture, which was analyzed as described. The amount of isoniazid was calculated by referring to the standard curve.

Analysis of Isoniazid Tablets—Twenty tablets were weighed and powdered, and the powder equivalent to 100 mg of isoniazid was weighed accurately. Four portions of 20 ml of ethanol were used to extract isoniazid from the tablet powder. Each extract was filtered[3], and the residue on the filter paper was washed with 10 ml of ethanol. The filtrate and

[1] Spectronic 20.
[2] E. Merck.
[3] Whatman No. 40 filter paper.

Table I—Analysis of Isoniazid and Its Hydrazones

Sample	Recovery, % USP Method[a]	Vanillin Method[b]	Proposed Method
Isoniazid	99.94 ± 0.52[c]	99.85 ± 0.62[c]	99.40 ± 0.76[c]
Glucose isonicotinoyl hydrazone	98.53 ± 0.59	—	—[d]
Lactose isonicotinoyl hydrazone	100.29 ± 0.68	—	—[d]
Isoniazid (50 mg) + glucose isonicotinoyl hydrazone (50 mg)	141.21 ± 0.85	—	99.45 ± 0.85
Isoniazid (100 mg) + glucose isonicotinoyl hydrazone (220 mg)	200.18 ± 0.86	105.80 ± 0.52	99.44 ± 0.87
Isoniazid (50 mg) + lactose isonicotinoyl hydrazone (100 mg)	159.40 ± 0.95	—	99.53 ± 0.76
Isoniazid (100 mg) + lactose isonicotinoyl hydrazone (350 mg)	205.61 ± 0.72	111.20 ± 0.81	99.62 ± 0.62

[a] Reference 16. [b] Reference 3. [c] Standard deviation was calculated from the results of 10 experiments. [d] Absorbance at 640 nm was insignificant.

Table II—Analysis of Isoniazid

Run	Recovery, % USP Method	Proposed Method
1	98.20	100.00
2	99.42	100.50
3	99.34	100.00
4	99.70	98.50
5	99.70	101.00
6	99.22	101.50
7	99.62	100.00
8	99.52	100.50
9	99.52	99.50
10	99.60	99.00
Mean	99.40	100.05
SD	±0.443	±0.91

washings were combined in a 100-ml volumetric flask and diluted to volume with ethanol.

The solution (10 ml) was diluted further with the same solvent to 100 ml and analyzed as described previously.

RESULTS

Concentration of 2,3-Dichloro-1,4-naphthoquinone—The absorbance at 640 nm of the colored product formed by the reaction of isoniazid (2 ml) and ammonia (6 ml) with 2,3-dichloro-1,4-naphthoquinone solution increased with an increase in the reagent concentration. The maximum absorbance was obtained in the presence of 10 ml of the reagent in 25 ml of the reaction mixture. On increasing the reagent quantity further, a slight decrease in intensity was observed (Fig. 2).

Concentration of Ammonia—A yellowish-green color was obtained on addition of 2,3-dichloro-1,4-naphthoquinone reagent (10 ml) to the mixture of isoniazid solution (2 ml) and ethanolic ammonia. Maximum color intensity was obtained in the presence of 6 ml of ammonia (10% w/v) in 25 ml of the reaction mixture (Fig. 3).

Temperature—The color intensity was maximum when the reaction mixture containing isoniazid solution (2 ml), alcoholic ammonia (6 ml),

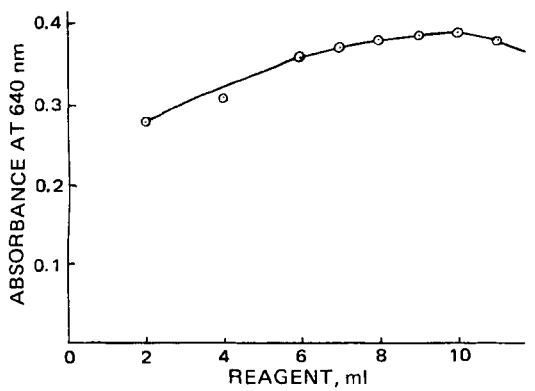

Figure 2—*Effect of reagent concentration on the absorbance at 640 nm.*

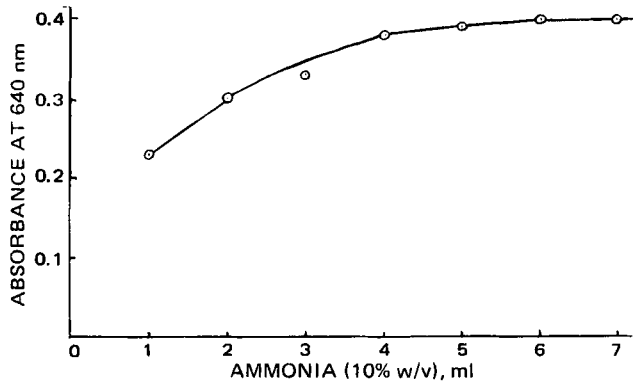

Figure 3—*Effect of ammonia concentration on the absorbance at 640 nm.*

and 2,3-dichloro-1,4-naphthoquinone reagent solution (10 ml) was kept in an ice bath for 25 min, and it remained constant on further standing. When the reaction was carried out at room temperature (25°), maximum color intensity was observed after 20 min; however, the color intensity obtained was low (Fig. 4).

Color Stability—Isoniazid solution (2 ml), ethanolic ammonia (6 ml), and 2,3-dichloro-1,4-naphthoquinone solution (10 ml) were reacted for 25 min in an ice bath. The reaction mixture was diluted to 25 ml with cold ethanol, and the flask was placed in an ice bath. After 5 min, the absorbance was measured at 640 nm. At ice bath temperature, the color intensity remained stable for more than 2 hr (Fig. 5).

Effect of Isoniazid Concentration—The absorbance at 640 nm was proportional to the amount of isoniazid in the concentration range of 1–14 μg/ml of the reaction mixture under experimental conditions.

DISCUSSION

The colored product obtained by reacting isoniazid with 2,3-dichloro-1,4-naphthoquinone in an aqueous basic medium was reported to be unstable (14). Therefore, the method was modified to increase the stability of the final colored product. Recently, a mechanism for the reaction between 2,3-dichloro-1,4-naphthoquinone and acid hydrazides was proposed (15). This method involves the reaction between isoniazid and 2,3-dichloro-1,4-naphthoquinone in an alcoholic medium containing ammonia. The colored product obtained has an absorbance maximum at 640 nm, and the color is stable for more than 2 hr.

Pure samples of isoniazid were analyzed by the USP (16) and the proposed methods. The results are in good agreement (Table II). Synthetic mixtures of isoniazid and its hydrazones were analyzed by the proposed procedure as well as the nitrite and vanillin methods (Table I). Both glucose isonicotinoyl hydrazone and lactose isonicotinoyl hydrazone were determined quantitatively by the official method (16) (Table I). No interference from hydrazones was observed in the proposed method, but the colorimetric procedure with vanillin gave higher recoveries for isoniazid in the presence of hydrazones (Table I). Although known to be imperfect, the vanillin method was employed in all earlier determinations of isoniazid in the presence of its hydrazones in biological fluids (6, 17). Because of the interference from hydrazones, a correction factor is used to compensate for the higher results obtained by this colorimetric method (17).

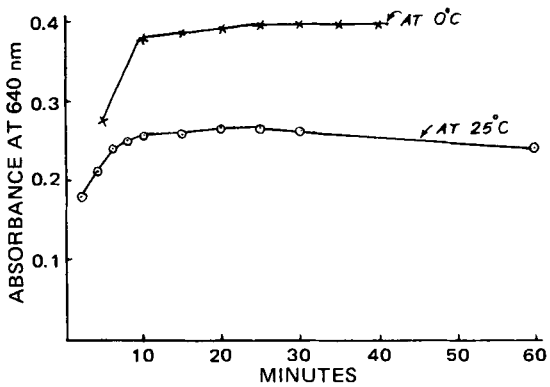

Figure 4—*Effect of time on absorbance at 640 nm.*

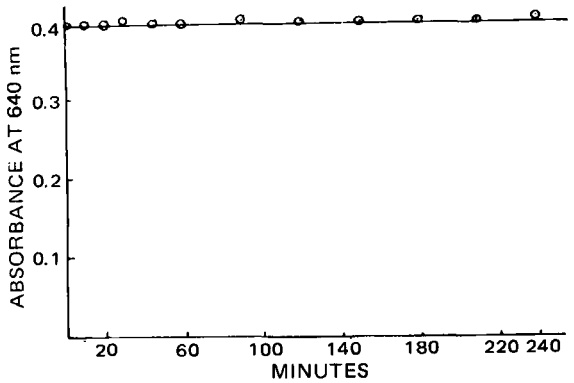

Figure 5—*Stability of colored product.*

For the present study, tablet samples were obtained from the local market. Five out of the eight samples were found by TLC to contain lactose and lactose isonicotinoyl hydrazone. Lactose-containing tablets (Table III, Samples A–D), when assayed by the proposed procedure, were found to contain between 78 and 90% of the labeled amount of free isoniazid. As much as 10–22% of isoniazid was present in the bound form with lactose and was probably not available for absorption. On the other hand, quantitative recoveries were obtained by the official method in the analysis of these tablets. Tablets that did not contain lactose gave comparable recoveries by both the official and the proposed methods.

When freshly prepared lactose-containing granules and the tablets prepared from them (Table III, Samples E and F) were analyzed by the proposed method, interaction between isoniazid and lactose was only 1–3%. Therefore, the significant interaction between isoniazid and lactose in the tablets apparently occurs only on standing over an extended period. The interaction seems to be negligible at the granulation stage.

The proposed method is specific for the estimation of isoniazid in the presence of its hydrazones. Significant interaction between isoniazid and lactose, resulting in the formation of lactose isonicotinoyl hydrazone, has been established. This interaction is likely to interfere with the bioavailability of isoniazid from its dosage forms.

Table III—Analysis of Isoniazid Tablets

Sample	Labeled Amount, mg/tablet	Recovery[a], mg/tablet: USP Method	Recovery[a], mg/tablet: Proposed Method	Presence of: Isoniazid Hydrazone	Presence of: Lactose
A	100	98.60	86.83	+	+
B	50	50.73	39.50	+	+
C	50	51.94	39.17	+	+
D	100	100.90	89.72	+	+
E[b]	300	305.21	301.20	+	+
F[c]	300	306.97	297.76	+	+
G	100	99.70	99.52	−	−
H	300	308.83	304.92	−	−
I	300	297.80	299.65	−	−

[a] Average result of three determinations. [b] Freshly prepared tablets. [c] Granules ready for the preparation of tablets.

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Mathematical Basis of Point–Area Deconvolution Method for Determining *In Vivo* Input Functions

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Abstract □ The point–area method for deconvolution derives a "staircase" input function which, when convolved onto the characteristic function, gives an output function coincidental with the given output data points. The area–area method for deconvolution is shown to be erroneous.

Keyphrases □ Deconvolution—point–area and area–area methods for determining *in vivo* input functions compared □ Input functions, *in vivo*—deconvolution point–area and area–area methods of determination compared □ Pharmacokinetics—deconvolution point–area and area–area methods for determining *in vivo* input functions compared

The use of *in vitro* dissolution functions for predicting differences in the rate and extent of *in vivo* drug dissolution depends on a correspondence (isomorphism) between the two processes. Any rigorous investigation of such an isomorphism ultimately requires the derivation of the *in vivo* drug input function. This function can be derived for