# Hydrazones of Isoniazid for Colorimetric Analysis

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**Abstract** □ Two sensitive color reactions of isoniazid are described. They are based on the formation of hydrazone Schiff bases when isoniazid is reacted with 4-nitrobenzaldehyde or pyridoxal. Linear curves were obtained with both reagents when absorbance values were plotted against concentrations.

**Keyphrases** □ Isoniazid—colorimetric analysis, hydrazones formed with 4-nitrobenzaldehyde or pyridoxal, prepared samples □ Colorimetry—analysis, isoniazid, hydrazones formed with 4-nitrobenzaldehyde or pyridoxal, prepared samples □ Antitubercular agents—isoniazid, colorimetric analysis, hydrazones formed with 4-nitrobenzaldehyde or pyridoxal, prepared samples □ Hydrazones—formed by reaction of isoniazid and 4-nitrobenzaldehyde or pyridoxal, colorimetric analysis

The USP XVIII (1) assay for isoniazid is based on iodometry. Some earlier reported spectrophotometric estimation methods involving color reactions due to Schiff base formation entailed reactions of isoniazid with benzaldehyde (2), vanillin (3), and 4-(dimethylamino)benzaldehyde (4). This report describes two color reactions based on the formation of yellow hydrazone Schiff bases when isoniazid is treated with 4-nitrobenzaldehyde or pyridoxal. Linear curves are obtained with both reagents by measuring the color intensities and plotting the absorbance values against graded concentrations of isoniazid.

## EXPERIMENTAL

**Reagents and Chemicals**—The following were used: 4-nitrobenzaldehyde solution, 0.4% (w/v), prepared by dissolving 4-nitrobenzaldehyde[1] (analytical reagent) in ethyl alcohol; pyridoxal hydrochloride solution, 0.25% (w/v), prepared by dissolving pyridoxal hydrochloride[2] (analytical reagent) in distilled water; and 1 *N* sodium hydroxide solution, prepared by dissolving sodium hydroxide[3] (analytical reagent) in distilled water.

**Standard Solution of Isoniazid**—Isoniazid[4] was dissolved in distilled water to give a 1-mg/ml solution.

**Determination of Wavelength of Maximum Absorption**—*Method A*—A 2.0-ml quantity of the standard solution of isoniazid (1 mg/ml) and 1.0 ml of 4-nitrobenzaldehyde solution (0.4% w/v) were mixed; after 10 min, the mixture was brought to volume (25 ml) with 1 *N* sodium hydroxide solution. After shaking the mixture well and allowing it to stand for 75 min, the yellow solution was scanned in the visible range using a spectrophotometer[5]. A broad peak was recorded between 409 and 430 nm. A blue filter (range of 400–530 nm) and a photoelectric colorimeter[6] were used for further analyses.

*Method B*—A 2.0-ml quantity of the standard solution of isoniazid (1 mg/ml), 6.0 ml of distilled water, and 2.0 ml of pyridoxal hydrochloride solution (0.25% w/v) were mixed in a glass-stoppered cylinder. After allowing the mixture to stand for 90 min, the yellow solution was scanned in the visible range using a spectrophotometer[5]. Although a well-defined peak could not be recorded, the maximum absorption was observed at 400 nm. The same blue filter and photoelectric colorimeter used in Method A were used for further analyses.

**Colorimetric Analysis with Graded Concentrations of Isoniazid**—4-Nitrobenzaldehyde solution, 1.0 ml (0.4% w/v), was added to each of seven volumetric flasks (25 ml) containing the standard solution of isoniazid as shown in Table I and treated as described for Method A. Similarly, 2.0 ml of pyridoxal hydrochloride solution (0.25% w/v) was added to each of five glass-stoppered cylinders (10 ml) containing the standard solution of isoniazid, diluted to volume with distilled water, and treated as described for Method B (Table II).

[1] B. D. H., England.
[2] E. Merck, Darmstadt, West Germany.
[3] Sarabhai M. Chemicals, Baroda, India.
[4] Pharmacopeial grade, Chemopharma Pvt. Ltd., Bombay, India.
[5] Beckman model DB.
[6] Systronix, type 101, Serial No. 149.

## DISCUSSION

The results given in Tables I and II indicate that the color reactions were sensitive at low concentrations of isoniazid and gave linear absorbance *versus* concentration curves with both chromogenic reagents. Color development in the isoniazid and 4-nitrobenzaldehyde system requires an alkaline medium.

**Table I—Concentration *versus* Absorbance for Isoniazid–4-Nitrobenzaldehyde Solutions**

| Number | Quantity of Standard Solution of Isoniazid, ml | Concentration of Isoniazid, mg/ml | Absorbance |
|---|---|---|---|
| 1 | 1.5 | 0.06 | 0.190 |
| 2 | 1.75 | 0.07 | 0.210 |
| 3 | 2.0 | 0.08 | 0.230 |
| 4 | 2.25 | 0.09 | 0.250 |
| 5 | 2.5 | 0.10 | 0.270 |
| 6 | 2.75 | 0.11 | 0.290 |
| 7 | 3.0 | 0.12 | 0.310 |

**Table II—Concentration *versus* Absorbance for Isoniazid–Pyridoxal Solutions**

| Number | Quantity of Standard Solution of Isoniazid, ml | Final Concentration of Isoniazid, mg/ml | Absorbance |
|---|---|---|---|
| 1 | 1.0 | 0.10 | 0.110 |
| 2 | 1.5 | 0.15 | 0.140 |
| 3 | 2.0 | 0.20 | 0.170 |
| 4 | 2.5 | 0.25 | 0.200 |
| 5 | 3.0 | 0.30 | 0.230 |

Both color reactions can be used for the estimation of isoniazid in pharmaceutical formulations. Examination of one commercial tablet preparation of isoniazid using both procedures afforded results comparable to those obtained by the USP XVIII procedure.

The hydrazone formed in the case of pyridoxal hydrochloride might conceivably be of therapeutic value in that it might not cause vitamin $B_6$ deficiency. In addition, it might possess lower toxicity and enhanced antituberculous activity when compared with isoniazid, as was reported for similar hydrazone compounds (5–7). The conversion of hydrazide to hydrazone apparently effectuates no loss in antituberculous activity.

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# Rapid Near IR Spectrophotometric Determination of Meprobamate in Pharmaceutical Preparations

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**Abstract** □ A rapid near IR spectrophotometric method was developed for determining meprobamate in tablets, sustained-release capsules, suspensions, and injectables. The absorbance of a chloroform solution of the drug is obtained at about 1.96 μm for quantitation. Assay of nine commercial products from four different manufacturers gave results ranging from 97 to 104% of label claim. Coefficients of variation of 0.7 and 1.3% were obtained on the tablets and a sustained-release product, respectively.

**Keyphrases** □ Meprobamate—IR spectrophotometric analysis, commercial dosage forms □ IR spectrophotometry—analysis, meprobamate, commercial dosage forms □ Sedatives—meprobamate, IR spectrophotometric analysis, commercial dosage forms

Since the USP XVIII (1) titrimetric procedure for determining meprobamate in tablets is lengthy and somewhat cumbersome, a rapid and easy-to-handle method is needed. A significant step toward this goal was taken by Sherken (2), who spectrophotometrically scanned chloroform solutions of the drug from 3.00 to 2.80 μm and utilized the absorbance at 2.91 μm to obtain the meprobamate concentration. This wavelength is characteristic of the symmetric stretching band of the primary amine group of meprobamate. However, for this procedure to be applicable, it was of paramount importance that the chloroform be freed of the stabilizer, alcohol. Therefore, extraction of the chloroform with water, followed by column chromatography through two tandem columns of activated alumina, was necessary.

Since the commencement of this work, USP XIX (3) adopted a colorimetric method for the assay of meprobamate in tablets. This method is sensitive and fairly rapid. However, the pH of the reagents must be carefully controlled and the acidified phenol reagent must be added carefully to decompose completely any chlorinating solution retained on the neck of the volumetric flask.

Because of the difficulties with these methods, a more rapid and facile procedure was desired that would be applicable to tablets and other dosage forms. The described near IR method is more rapid and does not suffer from the critical requirements of the other methods. Purification of the chloroform solution is not required since the alcohol does not interfere with the absorbance at 1.958 μm used to quantitate meprobamate. This band is subject to fewer interferences than the one at 2.91 μm (4). That the primary amine combination band at 1.958 μm is significantly weaker than the one at 2.91 μm also does not present any detection problem. High sensitivity is not of concern, because the levels of meprobamate in pharmaceutical preparations are generally high.

Table I—Comparison of Results Obtained on Commercial Products[a]

| Sample | Label Claim, mg | Near IR Found, mg | Near IR Label Claim, % | USP XIX Found, mg | USP XIX Label Claim, % |
|---|---|---|---|---|---|
| 1 (Tablet) | 400 | 408 | 102.0 | 388 | 97.0 |
| 2 (Tablet) | 400 | 403 | 100.8 | 393 | 98.3 |
| 3 (Tablet[b]) | 400 | 402 | 100.5 | 402 | 100.5 |
| 4 (Tablet) | 200 | 205 | 102.5 | 200 | 100.0 |
| 5 (Tablet) | 400 | 415 | 103.8 | 404 | 101.0 |
| 6 (Injectable[c]) | 400 | 394 | 98.6 | 400 | 100.0 |
| 7 (Capsule[d]) | 200 | 200 | 100.0 | 200 | 100.0 |
| 8 (Capsule[e]) | 300 | 295 | 98.3 | 355 | 118.0 |
| 9 (Suspension) | 200 | 194 | 97.0 | — | — |
| Mean | | | 100.4[f] | | 99.5[g] |

[a] Values reported are the average of duplicate assays. [b] Film-coated tablet. [c] Intramuscular injectable. [d] Sustained-release capsule. [e] Sustained-release capsule containing 15 mg of dextroamphetamine sulfate. [f] Samples 1–9. [g] Samples 1–7.

## EXPERIMENTAL

**Apparatus**—A recording spectrophotometer equipped with an IR source[1] and matching 5-cm silica cells were used.

**Reagents**—Chloroform (reagent grade) and a standard solution of meprobamate (NF XIV), 3.0–4.0 mg/ml in chloroform, were used.

**Sample Preparation**—*Tablets*—Determine the average tablet weight of not less than 20 tablets. Reduce the tablets to a fine powder and accurately weigh a portion, equivalent to 300–400 mg of meprobamate, into a 100-ml volumetric flask. Add about 70 ml of chloroform to the flask and shake for 15–20 min. Dilute to volume with chloroform and filter through paper[2], discarding the first 15–20 ml.

*Sustained-Release Capsules*—Determine the average net fill of not less than 20 capsules. Shake the contents to obtain a representative sample. Triturate an accurately weighed sample, equivalent to 300–400 mg of meprobamate; transfer it to a 100-ml volumetric flask with the aid of about 70 ml of chloroform and shake for 15–20 min. Dilute to volume with chloroform and filter as described for tablets.

*Suspensions*—Shake the sample for several minutes so that a representative sample aliquot can be removed. With a "to contain" pipet, transfer an aliquot equivalent to about 400 mg of meprobamate to a separator. Rinse the pipet thoroughly with distilled water into the separator and add water, if necessary, to obtain a volume of about 30 ml.

[1] Cary model 14.

[2] Whatman No. 1.