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# ANALYSIS OF ISONIAZID USING 2-METHYL-3-NITROPYRIDINE-6-CARBOXALDEHYDE

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## SUMMARY

The developed procedure is based on the interaction of isoniazid with 2-methyl-3-nitropyridine-6-carboxaldehyde at room temperature. The colour intensity of the reaction product was quantified spectrophotometrically at 385 nm with a lower limit of detection of 0.5 μg/ml. The colour is stable for at least 24 hours. There is no interference from congenial drugs, common carbohydrates and excipients used as additives in solid pharmaceutical formulations of isoniazid. The main degradation products of isoniazid do not interfere in the assay. The developed method can be recommended as a selective stability indicating assay.

## INTRODUCTION

The analytical procedures available for the assay of isoniazid include polarography (Turczan, 1967), coulometry (Wijnne et al., 1967), atomic absorption (Kidani et al, 1973), complexometry (Blagojević and Radosavljević, 1966; Spitzer et al., 1966), oxidimetry (Barakat and Shaker, 1966; Van Pinxteren and Verloop, 1964; Urbanyi and Winchurch, 1973), photoelectric colorimetry (Krishna et al., 1973), fluorimetry (Boxenbaum and Riegelman, 1974), ultra-violet (Dutt and Chua, 1964; Ghe and Peretti, 1968) and visible spectrophotometry (Akiyama et al., 1959; Kakemi et al., 1965; Nielsch and Giefer, 1959; Poole and Meyer, 1958; Scott, 1952; Stewart and Settle, 1975; Tan, 1973).

The colorimetric methods involve the interaction of isonicotinic acid hydrazide with different chromogenic agents. Among the chromogens used are those compounds containing labile halogen: 9-chloroacridine (Stewart and Settle, 1975), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Tan, 1973), 1-chloro- or 1-fluoro-2,4-dinitrobenzene (Poole and Meyer, 1958; Scott, 1952). Although highly sensitive and specific, these chromogens are skin irritant and produce relatively unstable colours.

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Estimation of isoniazid using the polymethine dye technique (Nielsch and Giefer, 1959) or its modified version for the assay of isoniazid and its metabolites in urine (Boxenbaum and Riegelman, 1974) has been reported and procedures involving the estimation of colour developed with 1,2-naphthaquinone-4-sulfonate and with phosphomolybdic acid have been described (Kakemi et al., 1965; Akiyama et al., 1959). Analysis of the drug and its metabolites in biological specimens has been carried out by measuring the developed colour of the hydrazones obtained from the interaction of vanillin or Ehrlich's reagent with isoniazid (Boxenbaum and Riegelman, 1974).

In the present investigation 2-methyl-3-nitropyridine-6-carboxaldehyde was studied as a sensitive and highly selective reagent for the estimation of isoniazid in pure form and in combination of other drugs in marketed pharmaceutical specialities. The developed method was further investigated as a stability indicating assay of isoniazid.

## MATERIALS AND METHODS

### *Intrumentation*

Spectra and absorbance measurements were made with a Speckol Spectrocolorimeter Carl Zeiss Jena spectrometer. Matched glass cells with 1 cm optical path were used. Infrared spectra were determined in potassium bromide pellets using a Beckman Spectrophotometer. Mass spectra were determined using 70 eV. (Analytical balance (WA-34, Poland) with 0.01 mg readability.)

### *Reagents and chemicals*

Isoniazid, streptomycin sulfate, pyridoxine hydrochloride, nicotinamide, sodium p-aminosalicylate, glucose, lactose, maize starch, gum acacia and magnesium stearate were pharmaceutical grade samples (B.P. 1973) and were used as supplied. Calcium p-benzamidosalicylate U.S.N.F. XIV grade was used. All solvents were reagent grade.

The colour reagent, 2-methyl-3-nitropyridine-6-carboxaldehyde, and the degradation products isonicotinamide and 1,2-bis(isonicotinoyl)hydrazine were synthesized according to the reported methods and were purified by distillation or double crystallization from an appropriate solvent.

### *Synthesis of colour reagent, degradation products and chromogen*

*2-Methyl-3-nitropyridine-6-carboxaldehyde* was prepared according to Banas and Shrowaczewska (1969) and purified by distillation at 4 mm Hg and 115°C to give faint yellow needles.

*Isinocotinamide* was prepared from ethylisonicotinate according to the method described by Hamer and Reynolds (1950) and was recrystallized from water to give pure colourless crystals, m.p. 161–162°C (reported 152–154°C).

*1,2-Bis(isonicotinoyl)hydrazine.* Isoniazid 6.0 g (0.04 mol), isonicotinoyl chloride hydrochloride 7.1 g (0.04 mol) and glacial acetic acid 25 ml were heated on a water bath for 1.5 h. After distillation of the solvent the residue was dissolved in water and neutralized with sodium carbonate. The residue formed was then filtered and purified by crystallization from water to yield colourless crystals, m.p. 270°C as reported (Byerman et al., 1954).

*Preparation and identification of the chromogen.* To a solution of isoniazid, 0.69 g (5 mmol) in 10 ml water, was added 0.6 ml 1 N sodium hydroxide and a solution of 0.83 g (5 mmol) of 2-methyl-3-nitropyridine-6-carboxaldehyde in 50 ml methanol. The mixture was left for 1 h and then filtered. The product was crystallized twice from 50% ethanol m.p. 210°C. Elemental microanalysis of the compound calculated according to the molecular formula $C_{13}H_{11}N_5O_3 \cdot H_2O$ was C: 51.49% required, 51.33% found; H: 4.29% required, 4.46% found; N: 23.1% required, 23.00% found.

The presence of characteristic bands for CONH (1685, 1545, 3450 $cm^{-1}$), $NO_2$ (1330, 845 $cm^{-1}$), and water of crystallization (3530, 1655 $cm^{-1}$) in the infrared spectrum of the compound provided further confirmation of the molecular structure II shown in Scheme 1. Molecular weight determination by mass spectrometry revealed a molecular ion peak at 285 m/e. $\epsilon_{max}$ was 385 nm in methanol 3705, and 20.52 X $10^3$ in methanol : water (9 : 1) in the presence of 0.1 ml of 1 N sodium hydroxide.

*Standard solution of isoniazid*

Weigh accurately 10 mg of isoniazid, transfer to 10 ml volumetric flask, and dilute to volume with distilled water.

*Colour reagent*

Weigh accurately 25 mg of 2-methyl-3-nitropyridine-6-carboxaldehyde, transfer to 50 ml volumetric flask, then dissolve and dilute to volume with methanol.

*Preparation of the assay solutions*

*For pure isoniazid.* Appropriate dilutions of standard solution of isoniazid are used as the assay solution.

*For synthetic mixture.* Transfer an accurately weighed amount of the powdered synthetic mixture equivalent to 1 mg of isoniazid in a 10 ml volumetric flask. Dissolve as completely as possible in distilled water. Filter, and discard the first portion of the filtrate. The clear solution obtained is the assay solution.

In the case of calcium p-benzamidosalicylate, 2 ml of the saturated aqueous solution is added to the synthetic mixture containing the equivalent of 1 mg isoniazid in a 10 ml volumetric flask. Dissolve as completely as possible in distilled water. Filter discard the first portion of the filtrate. The clear solution obtaines is used for the assay.

*For mixture containing the degradation products.* Accurately weigh on a watch glass 50 mg isoniazid, 44.8 mg isonicotinic acid, 44.5 mg isonicotinamide and 44.2 mg 1,2-bis-(isonicotinoyl)hydrazine; transfer quantitively to a 50 ml volumetric flask then dissolve and dilute to volume with distilled water.

*For tablets containing isoniazid.* Weigh and powder 20 tablets. Transfer an accurately weighed quantity of the powder equivalent to 50 mg of isoniazid in a 50 ml volumetric flask and proceed as under synthetic mixture starting from the dissolving (Table 2).

*For recovery determination.* Add an accurately weighed amount of isoniazid to an accurately weighed amount of the powdered tablets equivalent to a known amount of the isoniazid in a 50 ml volumetric flask, and proceed as under synthetic mixture starting from the dissolving. Tables 1 and 2.

*Development of colour.* Into 10 ml volumetric flasks each containing 1 ml of the

colour reagent pipette 1 ml of the assay solution of the appropriate dilution and 0.1 ml of 1 N sodium hydroxide; leave to stand for 20 min at room temperature with occasional shaking. Complete to volume with methanol and mix well. Determine the absorbance at 385 nm against the blank.

*Application of the developed method as stability indicating assay*

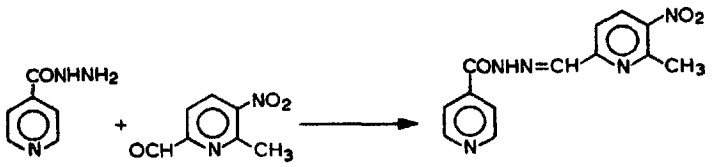
Prepare 0.2% solution of isoniazid in 0.1 N sodium hydroxide (pH 12). Titrate a 25 ml aliquot of this solution with standard bromine according to the B.P. 1973. For the colorimetric assay measure accurately 0.5 ml of the 0.2% isoniazid solution and dilute to 50 ml with distilled water. Take 1 ml from the diluted solution for the colour development and proceed as described above.

Repeat the analysis by both methods after standing at room temperature for 14 and 36 h.

## RESULTS AND DISCUSSION

The interaction between isoniazid and vanillin or Ehrlich's reagent to yield the respective coloured hydrazones was previously applied for the quantitative spectrophotometric determination of isoniazid (Boxenbaum and Riegelman, 1974). On the other hand, the successful application of 2-methyl-3-nitropyridine-6-carboxaldehyde (I) as an analytical reagent for the analysis of the hypotensive agent 1,4-dihydrazinophthalazine has been reported (Youssef et al., 1977). Initial attempts to react isoniazid with I yielded N-isonicotinoyl-2-methyl-3-nitro-6-pyridylformylhydrazone (II). The structure of the product II was identified by microanalysis, infra-red and mass spectrometric molecular weight determination. It revealed maximum absorbance at 385 nm with $\epsilon_{max}$ is methanol 3705 that was intensified on addition of sodium hydroxide to $20.52 \times 10^3$. Accordingly investigations were carried out to establish the most favourable conditions for the reaction illustrated in Scheme 1 and to achieve maximum colour development in the quantitative estimation of isoniazid.

SCHEME 1




I II

The influence of each of the following variables on the reaction was tested: pH of the reaction milieu, time of the reaction, temperature, concentration of reagent I, solvent, and wavelength at which maximum absorbance was reached.

No colour was developed at pH values below 7, and therefore different alkaline reaction catalysts were used: sodium hydroxide from 0.1 to 5 N, potassium carbonate 5%, and borax 0.1 N in aqueous solutions. The absorbance of the colour developed in the presence of sodium hydroxide was not appreciably affected by a change of normality and

was practically equal to that developed in the presence of potassium carbonate. However, in a milieu of borax the absorbance detected equalled one-half the value obtained in the presence of either sodium hydroxide or potassium carbonate solution.

Optimum reaction time was determined by estimating the colour intensity at room temperature (about 25°C), at 50°C and at the temperature of a boiling water bath. The results revealed that at room temperature the colour intensity became stable after 15 min and remained constant for at least 24 h. On the contrary, at 50°C a small decrease in absorbance was detected with time. The decrease in absorbance was potentiated on elevating the temperature of the reaction mixture up to that of a boiling water bath. This pattern can be attributed to the increased probability of degradation of isoniazid at elevated temperatures.

The optimum concentration of the colour reagent I was determined by adding 1 ml of varying dilutions of I in methanol (from 0.1 to 5 mg/ml) to a series of 10 ml volumetric flasks, each containing 1.0 ml of 2.0 μg isoniazid per ml and 0.1 ml of 1 N sodium hydroxide, and measuring the absorbance after 20 min at 385 nm against a blank and diluting each solution to volume with methanol. At reagent concentrations greater than 0.2 mg/ml, the colour intensity was only slightly dependent upon the reagent concentration. Therefore it was decided to use 0.5 mg/ml of the reagent as an optimum concentration for the assay and the standard calibration curve. The use of solvents other than methanol, e.g. ethanol or acetone, affected the absorbance adversely.

Beer's law was obeyed ($r = 0.9998$) up to an initial isoniazid concentration of 5 μg/ml ($A \approx 0.76$). A typical linear regression line has a slope of 0.1488, an intercept of 0.0189, and variance of 0.067.

*Quality control*

Isoniazid, an antitubercular drug, is usually formulated together with other antitubercular agents and/or vitamins in the presence of several frequently encountered excipients and additives. Therefore the influences of the ingredients listed in Table 1 were determined, keeping the ratios of concentration of the drugs and excipients relative to isoniazid in the simulated preparation within the same levels commonly found in the available marketed specialities. Table 1 illustrates the absence of interference of the drugs, the carbohydrates and the additives in the developed procedure. The standard deviation does not exceed 1.3 at the worst.

Commercial specialities were evaluated by comparing the proposed colorimetric method with the British Pharmacopoeia (1973) bromometric procedure. The data given in Table 2 illustrate the good agreement between the results of both procedures when applied to the analysis of formulae other than that including calcium p-benzamidosalicylate (formula D). The interference of this drug in the British Pharmacopoeia 1973 method was recognized by a more than double recovery of the calculated amount of isoniazid in addition to the non-reproducibility of the figures obtained.

Consequently the proposed spectroscopic method illustrates the validity of the reagent for the analysis of isoniazid in a wide variety of matrices. The method affords ease of analysis and reduction in analysis time in comparison with the suggested spectrophotometric or stoichiometric procedures, which demand the separation of isoniazid from p-aminosalicylic acid (Blagojević and Radosavljević, 1966; Dutt and Chua, 1964; Wijnne

TABLE 1

EFFECT OF VARIOUS DRUGS AND EXCIPIENTS ON RECOVERY OF ISONIAZID

| Substances added | Amount per 1 milligram of isoniazid | Recovery [b] % ± S.D. |
|---|---|---|
| Streptomycin | 4.0 | 99.76 ± 0.87 |
| Pyridoxine HCl | 0.5 | 98.70 ± 1.15 |
| Nicotinamide | 0.5 | 99.50 ± 0.92 |
| Sodium p-aminosalicylate | 40.0 | 100.00 (–) |
| Calcium p-benzamidosalicylate | – [a] | 100.00 (–) |
| Glucose | 5.0 | 99.73 ± 0.90 |
| Starch | 5.0 | 99.50 ± 1.30 |
| Sucrose | 10.0 | 99.80 ± 0.30 |
| Lactose | 200.0 | 99.76 ± 0.68 |
| Magnesium stearate | 20.0 | 99.70 ± 1.15 |
| Acacia | 20.0 | 101.10 ± 0.85 |

[a] Two milliliters of saturated aqueous solution.
[b] Average of three determinations.

et al., 1967) or from pyridoxine hydrochloride (Ghe and Peretti, 1968) by different partitioning techniques while maintaining similar precision and accuracy in addition to higher sensitivity.

*Stability study*

The degradation of isoniazid at room temperature and at pH 12 was investigated simultaneously by the colorimetric and the British Pharmacopoeia 1973 methods. The

TABLE 2

DETERMINATION OF ISONIAZID IN COMMERCIAL TABLET FORMULATIONS AND COMPARISONS WITH THE BRITISH PHARMACOPOEIA 1973 METHOD

A, Isoniazid 50 mg; B, isoniazid 250 mg, streptomycin 1 g; C, isoniazid 50 mg, pyridoxine HCl 5 mg, nicotinamide 5 mg; D, isoniazid 25 mg, pyridoxine HCl 5 mg, calcium p-benzamidosalicylate 1 g.

| Formula | Colorimetric method | | | Pharmacopoeial method | | |
|---|---|---|---|---|---|---|
| | Found [a] % ± S.D. | INH added (mg) | Recovery [a] % ± S.D. | Found % ± S.D. | INH added (mg) | Recovery [a] % ± S.D. |
| A | 86.00 ± 0.5 | 50 | 99.93 ± 1.2 | 85.94 ± 0.69 | 50 | 99.26 ± 0.057 |
| B | 97.05 ± 0.84 | 50 | 99.98 ± 1.3 | 97.31 ± 0.27 | 50 | 98.71 ± 1.2 |
| C | 91.1 ± 0.28 | 50 | 100.30 ± 0.43 | 92.10 ± 0.79 | 50 | 99.72 ± 0.95 |
| D | 101.75 ± 1.48 | 25 | 100.00 (–) | 277.19 Non-reproducible | – | – |

[a] Average of three determinations.

TABLE 3

EFFECT OF TIME ON THE STABILITY OF ISONIAZID AT pH 12

| Time (h) | Recovery % [a] | |
|---|---|---|
| | Pharmacopoeial method | Colorimetric method |
| 0 | 100.00 | 100.0 |
| 14 | 93.53 | 87.5 |
| 36 | 87.93 | 72.5 |

[a] Average of two determinations.

rather low selectivity of the British Pharmacopoeia 1973 bromometric method is shown by the pattern of higher results obtained relative to those of the proposed method, Table 3, since any degradation product susceptible to oxidation by bromine would probably interfere, meanwhile the proposed method allows the interaction with the intact hydrazide moiety.

For further confirmation a solution containing isoniazid together with equimolar ratios of the main degradation products was prepared (Kakemi et al., 1965, 1966) and analyzed by the developed method. It was noticed that isonicotinamide augments the solubility of 1,2-diisonicotinoylhydrazine to reach the given concentration ratio that was considered satisfactory for interference detection. Analysis of this solution by the developed method yielded quantitative recovery of isoniazid. This indicates the absence of interference by the degradition products. Consequently the colorimetric method can be recommended as a stability indicating assay.

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