Comparison of Analysis of Isoniazid and Its Dosage Forms by Several Methods

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Abstract ☐ Four assay procedures for determining isoniazid as the powder and in its dosage forms are compared. These include iodometric titration, bromometric titration, nitrite titration, and nonaqueous titration with acetous perchloric acid. The presence of reducing sugars interfered with the iodometric titration. The four methods do not distinguish isoniazid and its hydrazone derivative. However, the presence of hydrazone may be a serious problem since it may interfere with the bioavailability of isoniazid. Ferrocyphen was found suitable as an internal indicator in the nitrite titration method.

Keyphrases ☐ Isoniazid powder, tablets, and syrup—comparative analysis by iodine, bromometric, nitrite, and nonaqueous titration techniques, effect of sugars in formulation ☐ Titrimetry, iodine, bromometric, nitrite, and nonaqueous—analysis, isoniazid powder, tablets, and syrup, comparative study ☐ Sugars—effect on analysis of isoniazid syrup ☐ Ferrocyphen—evaluation as internal indicator in nitrite titration for isoniazid analysis

Isoniazid and its dosage forms have been official since USP XV. The assay procedure has always involved the addition of an excess of standard iodine solution in the presence of sodium bicarbonate. After a definite standing period, the excess iodine is titrated with standard thiosulfate. The method was first proposed by Canback (1) who analyzed isoniazid powder and commercial tablets of isoniazid. At about the same time, a similar method was described (2) in which sodium hydroxide was used in place of sodium bicarbonate. Several workers (3–5) reported high results with the official procedure because of interference by excipients such as lactose and glucose, which are reducing sugars and susceptible to oxidation by iodine. Also, low results have been noted[1] with the drug entity (3, 6).

The BP (7) recognizes isoniazid and the tablet dosage form. The assay procedure involves residual bromometric titration. Excess bromine is determined by titration with thiosulfate solution. A direct visual bromometric titration procedure was reported (8), and a potentiometric bromometric titration method was described (9). The bromometric method was reported (6) to give results that were 0.5% high. However, the BP method for the drug entity and the tablet dosage form was reported (3) to be reliable, consistent, and superior to the iodine titration procedure.

A nitrous acid titration method was reported (10) using standard sodium nitrite solution as the titrant in the presence of dilute hydrochloric acid, with starch iodide as an external indicator. This is the method described in the USP (11) for the nitrite titration of sulfa drugs. Noxon (5) described a potentiometric titration procedure for analyzing isoniazid tablets based on the nitrite titration procedure for sulfonamides. This has been adapted as a semiautomated procedure by the National Center for Drug Analysis.

Nonaqueous titrimetric procedures have been suggested by several workers (2, 6, 10, 12). The titrant was either perchloric acid in acetic acid or perchloric acid in dioxane. Titrations were performed visually with crystal violet as the indicator or potentiometrically using a glass–calomel electrode system. The titration solvent was an acetonitrile–acetic anhydride mixture, acetic acid, acetic anhydride, or a mixture of acetic acid and acetic anhydride, and these methods have been reviewed (13).

Since 1952, various other methods, *e.g.*, colorimetry, spectrophotometry, titrimetry, and oxidation–reduction methods, have been proposed for the determination of isoniazid and its dosage forms, alone and combined with other therapeutic agents (4, 14). Wu *et al.* (15) investigated the solid-state interaction between isoniazid and the adjuvants magnesium oxide and lactose since it was felt that such solid–solid interactions may adversely affect drug bioavailability. Diffuse reflectance spectroscopic studies indicated that chemisorption and physical adsorption of isoniazid on magnesium oxide did take place. The browning reaction between isoniazid and lactose was also studied by this technique. Hydrazone formation between lactose and isoniazid was detected by TLC. These interactions between isoniazid and lactose apparently do not proceed to any great degree in the dry solid state at ambient temperatures; but in the presence of moisture or high humidity and at elevated temperatures over an extended period, hydrazone formation and the browning reaction could become significant factors.

In liquid dosage forms such as syrups, hydrazone formation is a distinct probability where a reducing sugar is used in the formulation. Rao *et al.* (16) found that in commercial syrups containing isoniazid, as much as 60–70% of the isoniazid became bound as the hydrazone within 24 hr. Studies involving human subjects indicated that absorption of isoniazid from such syrups was severely impaired as a result of this condensation. Furthermore, from a 6-hr absorption study in humans, it appeared that the bound isoniazid was not absorbed nor was there any indication that the bound form was converted to free isoniazid in the stomach or gut. The investigators recommended that sugars including glucose, fructose, and sucrose (though not a reducing sugar *per se*) not be employed in the formulation of isoniazid

[1] J. A. Mollica, Ciba-Geigy Corp., personal communication.

Table I—Analysis of Isoniazid Powder and Its Dosage Forms

Dosage Form	Method of Analysis: USP	Nitrite	BP	Nonaqueous
Powder	99.05 ± 0.22[a]	100.03 ± 0.45[b] 100.48 ± 0.28[c]	98.56 ± 0.66	100.46 ± 0.67
Tablets	95.95 ± 0.38	97.96 ± 0.41 95.01 ± 0.20	97.79 ± 0.84	95.78 ± 0.45
Syrup	98.09 ± 0.53	103.07 ± 1.22 102.36 ± 0.62	100.10 ± 0.22	97.53 ± 0.84

[a] Percent recovery based on at least four determinations ± *SD*. [b] Starch iodide indicator. [c] Ferrocyphen indicator.

Table II—Effect of Glucose, Sucrose, and Lactose on Analysis of Isoniazid

Sugar Content, % (w/v)	USP Method Recovery, %		BP Method[a] Recovery, %	Nitrite Method[a] Recovery, %
	No Isoniazid	Isoniazid	Isoniazid	Isoniazid
Glucose				
10	57.61 ± 1.15	113.75 ± 1.38	99.99 ± 0.32	99.92 ± 0.84
20	67.89 ± 0.80	121.41 ± 2.39	99.57 ± 0.44	99.70 ± 1.26
30	75.16 ± 0.97	138.25 ± 0.73	98.63 ± 0.44	100.24 ± 0.54
40	78.50 ± 1.59	135.88 ± 2.52	97.23 ± 1.55	101.09 ± 0.12
50	82.07 ± 0.16	136.34 ± 0.32	98.19 ± 0.98	99.43 ± 0.54
Sucrose				
10	0.31 ± 0.23	98.40 ± 0.09	97.56 ± 0.62	99.47 ± 0.57
20	0.80 ± 0.31	98.42 ± 0.22	97.10 ± 0.33	99.69 ± 0.18
30	1.11 ± 0.17	98.56 ± 0.13	97.42 ± 0.07	99.38 ± 0.33
40	1.12 ± 0.06	98.14 ± 0.10	98.40 ± 0.44	98.84 ± 0.31
50	1.11 ± 0.07	98.44 ± 0.09	97.51 ± 0.20	100.10 ± 0.36
Lactose				
10	49.59 ± 1.01	105.37 ± 0.23	94.34 ± 0.31	98.80 ± 0.10
20	63.65 ± 2.67	110.33 ± 0.66	95.33 ± 0.38	97.89 ± 0.30

[a] The percent recovery was 0.00 in the absence of isoniazid.

syrup. Sorbitol, however, appears to be quite acceptable.

In the present study, a comparison is made of the four major methods of assay used for determining isoniazid as the powder and in its dosage forms. These include the iodine titration procedure (USP XVIII), bromometric titration (BP 1968), nitrite titration (proposed for adoption in USP XIX), and nonaqueous titration with perchloric acid. Also included in this study is the effect of the presence of certain sugars on the analytical procedure. Ferrocyphen is evaluated as an internal indicator in the nitrite titration method.

EXPERIMENTAL

Methods of Analysis—*Iodine Titration*—Isoniazid powder and the tablet and syrup dosage forms were analyzed by the procedure described in USP XVIII (11).

Bromometric Titration—Isoniazid powder and tablets were analyzed according to the method described in the BP (7). Isoniazid syrup USP was analyzed by the procedure described in the BP for the tablet dosage form. A 5.0-ml aliquot of the syrup was treated in a similar manner as the aliquot of the powder mass described in the BP assay.

Nitrite Titration—A combination of the procedure described in USP XVIII (11) and that of Noxon (5) was employed. About 100 mg of isoniazid, accurately weighed, was dissolved in 50 ml of a solvent containing 10% potassium bromide in 2 *N* HCl contained in a 100-ml beaker. For the syrup, an accurately measured volume equivalent to 100 mg of isoniazid was taken for analysis. For the tablet dosage form, 20 tablets were weighed and reduced to a fine powder and an aliquot of the powder mass equivalent to 100 mg of isoniazid, accurately weighed, was taken for analysis. The syrup aliquot or the aliquot of the powdered tablet mass was dissolved in 50 ml of 10% potassium bromide in 2 *N* HCl. The procedure then followed was that described in the USP (11) under nitrite titration beginning with "cool to 15°."

Table III—Analysis of 1-Isonicotinyl-2-D-glucosylhydrazine

Method	Recovery, %
Nitrite	99.71 ± 0.93
USP	103.92 ± 1.20
BP	99.32 ± 0.45
Nonaqueous	100.31 ± 0.17

A series of nitrite titrations was performed on isoniazid and its dosage forms using ferrocyphen indicator solution. The indicator solution was prepared as directed by Banick and Valentine (17). Titrations were conducted as referred to previously, except 1 ml of indicator solution was added to the titration solution in place of using starch iodide as an external indicator. Solutions were titrated to a violet end-point which persisted for at least 3 min.

Nonaqueous Titration—Isoniazid powder was analyzed by transferring about 100 mg of powder, accurately weighed, to a 100-ml beaker. The powder was dissolved in 50 ml of glacial acetic acid or a mixture of acetonitrile-acetic anhydride (1:1). The solution was titrated potentiometrically with 0.1 *N* perchloric acid in glacial acetic acid or dioxane, prepared and standardized as directed in the USP (11). Titrations were performed with a titrimeter[2] equipped with a calomel-glass electrode system. The calomel electrode was modified by replacing the aqueous bridge in the calomel cell with a saturated solution of potassium chloride in methanol. Increments of 0.1 ml of titrant were added in the vicinity of the end-point. The exact end-point was determined from the inflection in the curve obtained by plotting millivolts *versus* milliliters of titrant added.

Isoniazid tablets were analyzed nonaqueously by weighing and finely powdering 20 tablets. An aliquot of the powder equivalent to about 500 mg of isoniazid, accurately weighed, was treated in

[2] Fisher model 35.

the same manner as described under isoniazid tablets in the USP (11) up to the point where the directions state: "Pipet 20 ml. of the methanol solution into a beaker." This solution was evaporated to dryness, the residue was dissolved in 50 ml of glacial acetic acid or a mixture of acetonitrile–acetic anhydride (1:1), and this solution was titrated nonaqueously as described earlier.

Isoniazid syrup was analyzed by transferring a 5.0-ml aliquot to a beaker and diluting to about 50 ml with water. The solution was transferred quantitatively to a chromatographic column containing a strong cation-exchange resin[3]. The resin column was prepared and treated as described earlier (18). When the last of the diluted syrup solution just disappeared below the resin surface, water was added to the column until at least 500 ml of eluate was collected. The eluate contained sugar and other nonionic components of the syrup. The column was then eluted with 60 ml of 10% ammonium hydroxide in alcohol. The eluate was evaporated to dryness, the residue was dissolved in 50 ml of glacial acetic acid, and the solution was titrated potentiometrically with 0.1 *N* perchloric acid as described previously.

Interference by Hydrazone Formation—Since isoniazid reacts with reducing sugars to form an isonicotinyl hydrazone, the effect of hydrazone formation on the analysis for isoniazid was studied. 1-Isonicotinyl-2-D-glucosylhydrazine was synthesized according to the method of Fox (19). Its identity and purity agreed with those of the known compound.

The product, crystallized from absolute methanol, melted at 159–161° [lit. (19) 160° dec.]. It showed IR absorption maxima (mineral oil) at 3550–3450 (OH), 1668 (C=N), 1580 (CONH), and 1375 (CH_2OH, CHOH) cm^{-1}. Elemental analysis for carbon, hydrogen, and nitrogen content of a sample (98% purity) gave the following results:

Anal.—Calc. for $C_{12}H_{17}N_3O_6$: C, 48.16; H, 5.73; N, 14.04. Found: C, 48.18; H, 5.95; N, 13.89.

1-Isonicotinyl-2-D-glucosylhydrazine was analyzed by the four procedures described earlier.

Interference by Sugars—A series of solutions was prepared containing the following sugars: sucrose, 10, 20, 30, 40, and 50%; glucose, 10, 20, 30, 40, and 50%; and lactose, 10 and 20%. Each solution contained 10 mg isoniazid/ml. A similar series of the solutions contained no isoniazid and served as controls. All solutions were analyzed by the iodometric, bromometric, and nitrite titration procedures for isoniazid content as described under the specific procedure.

RESULTS AND DISCUSSION

The analysis of isoniazid as the drug entity, in dosage forms, and in combination with other drugs has been the subject of extensive study over the past 20 years. While many procedures have been proposed for the assay, the USP and BP have only recognized the iodometric method and the bromometric procedure, respectively. The present study was undertaken because of shortcomings reported for the iodometric method. As a result of this investigation, the nitrite titration procedure has been recommended for adoption by the USP for isoniazid and its dosage forms.

Four methods of assay for isoniazid and its dosage forms were subjected to a comparative study (Table I). No significant difference was noted in the analysis of the powder by the four procedures. The iodometric, nitrite, and bromometric methods were rapid and accurate, producing readily discernible end-points and quantitative recovery data with good precision. The nonaqueous procedure did not permit a suitable visual titration, although satisfactory results were obtained by potentiometric titration. However, *this is time consuming* since plotting of data is required for end-point determination. Consistent results were obtained for the analysis of the tablet dosage form by the four methods. Data are also reported in Table I for the analysis of a commercial syrup containing isoniazid.

Difficulties were encountered when the nonaqueous titration procedure was applied to the analysis of the syrup dosage form. In a preliminary study, an aliquot of the syrup was heated at 70–80° until all volatile material was driven off. The thick semisolid residue was dissolved in glacial acetic acid and titrated potentiometrically with acetous perchloric acid. No detectable end-point was obtained. The presence of sugars apparently interferes and prevents successful nonaqueous titration. This problem was resolved by passing the syrup aliquot through a cation-exchange resin which extracted the isoniazid. Washing the column thoroughly with water assured removal of the sugar and other nonionic components. Elution of the isoniazid with ammonium hydroxide in ethanol and subsequent evaporation of the solvent produced pure isoniazid which was then titrated nonaqueously. While quantitative recovery data were obtained, the method was overly time consuming and burdensome.

The nitrite titration procedure produces equally satisfactory results whether end-point detection is effected potentiometrically, using a saturated calomel–platinum electrode system, or visually, using starch iodide paper as an external indicator or ferrocyphen indicator solution as an internal indicator.

It was noted earlier (3–5), and confirmed by supplied data[4], that certain sugars interfere in the analysis of isoniazid by the USP XVIII method. A systematic study was undertaken to determine the extent of interference by glucose, sucrose, and lactose in the assay for isoniazid (Table II). The poor solubility of lactose permitted the study of only two concentration levels.

Wu *et al.* (15) and Rao *et al.* (16) showed that glucose and lactose may form a hydrazone with isoniazid in solid and liquid dosage forms. It was of interest to determine whether hydrazone formation does, in fact, interfere with the official assay for isoniazid. Also of concern was whether reducing sugars would interact with the iodine solution titrant, thus causing high results. It is apparent from the data in Table II that sugars in the absence of isoniazid do react positively to the USP assay for isoniazid. Even sucrose, which is not a reducing sugar, does titrate in high concentrations with iodine solution, although interference in the assay in the presence of isoniazid was not perceptible. No interference by sugars was noted with the bromometric or nitrite procedures. Titrant was not consumed in the absence of isoniazid, and quantitative recoveries are reported for syrups containing isoniazid.

The isonicotinyl hydrazone of glucose was synthesized and characterized as described under *Experimental.* The hydrazone was assayed by the four procedures studied (Table III). The official method produced results that were somewhat higher than theory. The iodine may have interacted with glucose formed by hydrolysis of the hydrazone during the assay procedure. This problem was not pursued. If hydrazone formation does occur in the tablet or syrup dosage form, interference with the nitrite, BP, and nonaqueous procedures would not be expected; *i.e.*, quantitative recovery for isoniazid would be obtained whether the isoniazid was free or combined as hydrazone. In other words, these methods do not distinguish isoniazid from its condensation product. It was shown earlier that the official method cannot be used in the presence of reducing sugars. Although hydrazone formation does not interfere with the three methods indicated, its presence may represent a more serious problem in terms of the interference with the bioavailability of isoniazid, as noted by Rao *et al.* (16). This aspect of the problem was beyond the scope of this study.

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PHARMACEUTICAL TECHNOLOGY

Automated Fluorescence Analysis of Perphenazine in Dissolution Rate Testing of Tablet Formulations

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Abstract □ An automated fluorescence method is described for determining perphenazine in large numbers of samples, as might be required in dissolution studies. Tablets containing perphenazine and amitriptyline were dissolved in 0.1 *N* HCl. Approximately 5 ml was transferred to the automated system where amitriptyline was removed as the ion-pair by extraction with chloroform and discarded. Perphenazine in the aqueous phase was reacted with permanganate solution to form a fluorophore which is quantitative. No sample blank was required. Analyses may be carried out at a rate of 30/hr. Sensitivity was approximately 0.1 μg/ml with a relative standard deviation of ±1.7%.

Keyphrases □ Perphenazine from perphenazine–amitriptyline tablets—automated fluorescence analysis in dissolution studies □ Dissolution rate testing of perphenazine–amitriptyline tablets—automated fluorescence analysis of perphenazine □ Fluorometry—analysis, automated, perphenazine from perphenazine–amitriptyline tablets during dissolution studies

A procedure based on the reaction between palladium (II) chloride and certain phenothiazines, including perphenazine, was reported previously (1, 2). This procedure was shown to be specific for perphenazine in the presence of certain oxidative degradates, specifically the sulfoxide. However, some interferences were encountered when applying this procedure to determinations of perphenazine. The interferences were due to excipients and could be avoided only by employing sample blanks. Furthermore, the palladium chloride assay was not sensitive enough to determine the low levels of perphenazine found in dissolution studies (0.5–5 μg/ml).

Mellinger and Keeler (3) described the identification of some phenothiazine drugs, including perphenazine, by monitoring the fluorescence obtained after reacting the drugs with potassium permanganate. This procedure has been automated and applied to the determination of perphenazine in dissolution studies of perphenazine–amitriptyline[1], a tranquilizer–antidepressant combination, in various proportions. Amitriptyline was removed by extraction prior to the permanganate reaction because it (or the oxidative products) interfered with the fluorescence of perphenazine. No sample blank was required.

EXPERIMENTAL

Materials—The following were used: chloroform, hydrochloric acid (0.1 *N*), and potassium permanganate solution (0.01% in 0.1 *N* HCl). Methanol and 0.1 *N* HCl served as purge solutions. All materials were reagent grade.

Apparatus—The analytical train consisted of the following: a liquid sampler II[2] and a proportioning pump[2], model I; a recording fluorescence spectrophotometer[3] equipped with a ratio mode and a flow cell (0.5-ml capacity and designed to measure fluorescence at an angle of 90° to the excitation beam); and a recorder[4].

[1] Triavil, Merck & Co.
[2] Technicon Corp., Tarrytown, N.Y.
[3] MPF-2A, Perkin-Elmer Corp., Norwalk, Conn.
[4] Sargent model SRL.