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Short communication

Stability of isoniazid in isoniazid syrup: formation of hydrazine

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1. Introduction

Renewed industry interest in marketing liquid isoniazid formulations prompted us to review the current USP monograph for isoniazid syrup [1]. Surprisingly, the assay for the active in the product is not stability indicating, consisting simply of a sodium nitrite titration which indiscriminately quantitates an amine function by diazonium salt formation. Hydrazine, one of isoniazid's principal degradation products (Fig. 1), is also an amine and thus is not distinguished from parent isoniazid. Hydrazine is a known carcinogen [5] and considerably more toxic than isoniazid an LD_{50} in mice of 149 mg kg^{-1} i.v. for isoniazid but only 57 mg kg^{-1} i.v. for hydrazine [2]. Isoni-

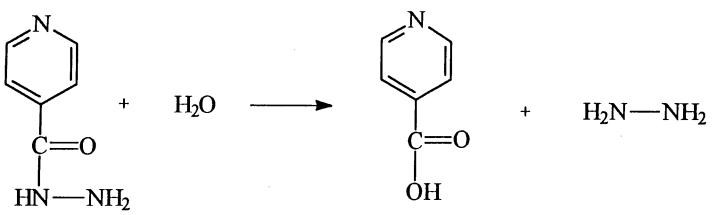

Fig. 1. Degradation of isoniazid to isonicotinic acid and hydrazine.

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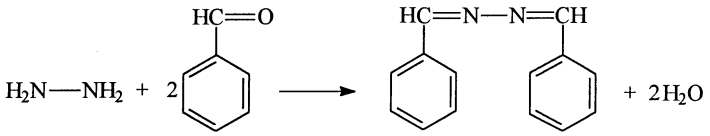

Fig. 2. Reaction of hydrazine with benzaldehyde to form benzalazine.

azid itself has been reported to be carcinogenic in mice [3] but the carcinogenic activity is probably due to the release of free hydrazine. Supporting this is the fact that: (i) ethionamide, structurally closely related to isoniazid but lacking the ability to split off hydrazine, is not carcinogenic in BALB/c mice; and (ii) 4-(isonicotinylhydrazine)pimelic acid which does release hydrazine but with greater difficulty than isoniazid has a lower carcinogenicity than isoniazid [4]. Since isoniazid remains a primary drug for the treatment of all types of tuberculosis [5] and is normally given in high doses over long periods of time (300 mg day^{-1} for 6–9 months) [6], the inadequacy of the current compendial assay in failing to distinguish between isoniazid and hydrazine prompted our concern.

We assessed the stability of a commercial isoniazid syrup formulation under several different storage conditions over a period of 4 months by following the formation of hydrazine. To determine hydrazine concentration, we adapted a published method [7] which quantitates hydrazine by reacting it with benzaldehyde to form benzalazine as shown in Fig. 2. The benzalazine is then determined by gas chromatography. Isoniazid also reacts with benzaldehyde but the reaction product does not elute in our chromatographic system thus enabling trace levels of hydrazine to be quantitated in the presence of a large excess of isoniazid. We achieved a lower limit of quantitation of 10 ppm, based on the lowest standard run. The standard curves were linear from 10 to 100 ppm.

2. Experimental

2.1. Reagents and supplies

Glass vials (we used 20 ml scintillation vials), benzaldehyde, D-sorbitol (97%), 3-chloroacetophenone and benzalazine were obtained from Aldrich Chemical (Milwaukee, WI). Hydrazine hydrate and Nalgene® (HDPE) 30 ml narrow mouth bottles were obtained from Sigma (St. Louis, MO). Heptane, HPLC grade, was obtained from Burdick and Jackson (Muskegon, MI). Qorpak® phenolic plastic caps, lined with a conical polyethylene liner, were obtained from Thomas Scientific (Swedesboro, NJ). All reagents were used without further purification. A Sybron-Barnstead (Dubuque, IA) Thermolyne Speci-Mix Model M-26125 rocker was used for sample extractions.

2.2. Preparation of samples for stability study

An amount of 5.0–5.2 g of simulated commercial isoniazid syrup (50 mg 5 ml^{-1}) was transferred to each of 36 glass vials (20 ml capacity) and 36 Nalgene® (HDPE) bottles (30 ml capacity). Equal numbers of glass vials and HDPE vials were placed in a refrigerator at 0°C, ambient temperature (23–27°C) and an oven maintained at 40°C. All were protected from light. At periodic intervals, a glass bottle and an HDPE bottle were withdrawn at each of the temperature stations and assayed.

2.3. Preparation of standards in simulated product vehicle

Standard solutions of hydrazine in a 70% sor-

bitol vehicle (simulating the commercial product) were prepared by spiking ca. 1.3 g of the vehicle with aliquots of a freshly prepared standard solution of hydrazine hydrate in water (ca. 1.0 μg μl^{-1}) to give standard levels of 10, 25, 50 and 100 ppm. New standards were prepared each day the assay was run. The vehicle without added hydrazine was assayed as a blank.

2.4. Assay procedure

Syrup (ca. 1.3 g) was weighed into a glass tube ca. 10 cm × 1.2 cm and 2.0 ml of water, 50 μl of a 0.4-ml ml^{-1} solution of benzaldehyde in methanol was added, the tube was capped with a phenolic resin cap lined with a conical polyethylene liner, and the tube rocked on a Speci-Mix rocker for 0.5 h. A 200-μl aliquot of internal standard solution (3-chloroacetophenone, 0.05 mg ml^{-1} in heptane) was then added and the tube rocked on the Speci-Mix for an additional 15 min to extract the reaction product into the heptane phase. (The internal standard must be added after the derivatization has taken place to eliminate the possibility of reaction between hydrazine and the internal standard which is also a carbonyl compound). The tube was centrifuged at 2000 rpm for 10 min to completely separate the phases and a portion of the heptane extract was injected into the gas chromatograph.

2.5. Chromatographic conditions

Gas chromatographic analyses were carried out with a Hewlett-Packard 5890 GC equipped with a DB-5 fused silica capillary column (15 m × 0.32 mm i.d. × 0.25 μ film thickness; J&W Scientific, Folsom, CA). Detection was by electron capture (Ni^{63}). Although electron capture did not afford increased sensitivity over flame ionization, we felt its increased selectivity would reduce the chance of interferences due to formulation variations when the method was applied to commercial products. The temperatures of both the detector and the injection port were 315°C. The column head pressure was 10 psi. The injection volume was ca. 0.3 μl and made in splitless mode with a splitless sampling time of 0.5 min. After injecting the sample, the oven temperature was held at 65°C for 0.5 min and then increased at 12°C min^{-1} until the hydrazine (as benzalazine) peak eluted. The total run time was ca. 10 min.

2.6. Calculations

Peak areas, peak area ratios and retention times were determined using a Hewlett-Packard 3396A integrator. From the peak area ratios and known hydrazine concentrations of the standards, a regression line was plotted using Sigma-Plot® software. From the slope and intercept of the regression line, the concentration of hydrazine in the stability samples was determined.

3. Results

The concentrations of free hydrazine found versus time are presented in Table 1. Typical chromatograms obtained from stability samples at 0°C, ambient temperature and 40°C stored in glass are shown in Fig. 3. Using the above conditions, the observed uncorrected retention times were: 3-chloroacetophenone (internal standard): 7.8 min, hydrazine (as benzalazine): 9.2 min (the identity of the benzalazine peak was confirmed by injection of a dilute solution of commercial reagent grade benzalazine in heptane). The chromatograms obtained from samples stored in HDPE bottles, not presented here, did not differ significantly from those obtained from the samples stored in glass. While the benzalazine peak

Table 1
Hydrazine in isoniazid syrup, ppm

Time, days	0°C	Ambient	40°C
0	BDL, BDL	BDL, BDL	BDL, BDL
12	BDL, BDL	1.6, 3.9	7.0, 7.4
32	BDL, BDL	2.4, 3.9	16, 16
53	TRACE, BDL	2.5, 4.4	27, 32
84	BDL, BDL	7.6, 6.0	43, 45
123	BDL, BDL	12, 13	65, 62

First result at each time point is storage in glass; second result is storage in HDPE bottle. Results below 10 ppm are estimates. BDL—below detectable level.

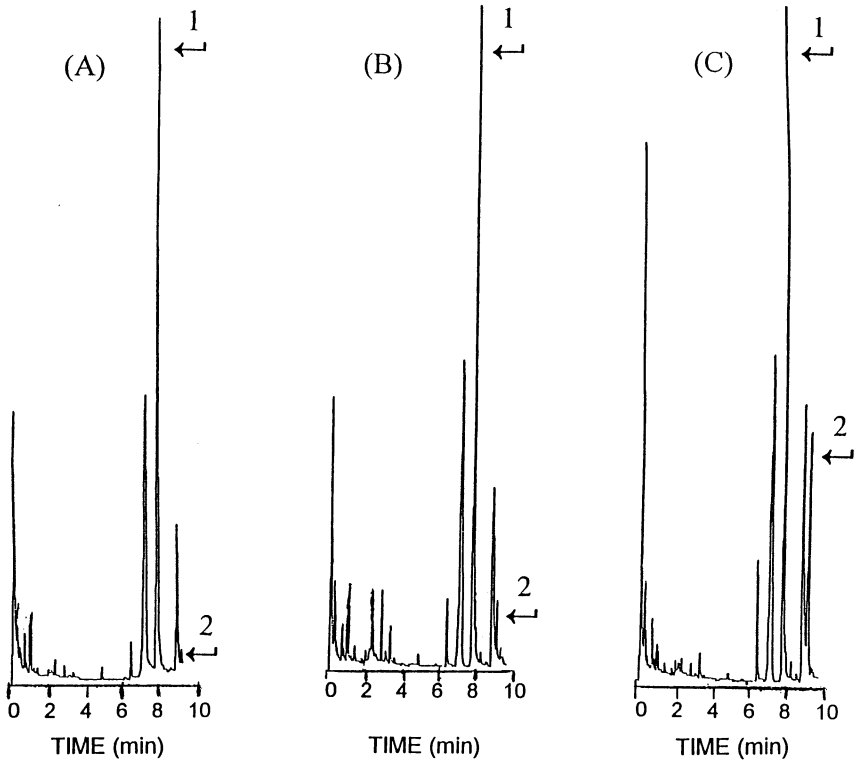

Fig. 3. Typical chromatograms: hydrazine (as benzalazine) in isoniazid syrup. Syrup stored for 4 months at: (A) 0°C; (B) ambient temperature (23–27°C); and (C) 40°C. Peak 1: internal standard (3-chlorobenzophenone), ret. time 7.8 min. Peak 2: hydrazine (as benzalazine), ret. time 9.1 min.

was not base-line separated from a closely eluting endogenous peak ($\alpha = 1.04$), the chromatographic selectivity was nevertheless sufficient to allow adequate peak area integration. The average inter-day CV as measured by the reproducibility of the slopes of the standard curves ($n = 6$) was 15%. We determined intra-day precision by repetitive assay of 25 and 100 ppm standards ($n = 4$). The CVs were 9.5 and 11.3%, respectively. A typical regression line is presented in Fig. 4.

At 0°C, no hydrazine was detected. Decomposition to hydrazine was observed at ambient temperature with a 5.5–6.0 × increase in decomposition rate when the storage temperature was raised to 40°C as measured by the slopes of the hydrazine formation curves. Formation of hydrazine was linear with time. The concentrations found are presented in Table 1 and plotted in Fig. 5.

4. Conclusions

It has been reported that administration of hydrazine sulfate at ca. 11.2 ppm (equivalent to 2.76 ppm free hydrazine) per day to CBA mice for 25 weeks caused a statistically significant increase in the incidence of hepatocarcinomas in male mice (28% vs. 10% in controls) [8]. Linear extrapolation using the Gaylor-Kodell method developed by the National Center for Toxicological Research indicates that the safe exposure level for hydrazine—defined as the threshold above which there is a one in one million increase in the risk of developing a tumor over one's lifetime is 77 ppt [9].

We have shown that levels of hydrazine ranging from 1.6 to 12.6 ppm are found in isoniazid syrup stored at ambient temperature for 4 months while in contrast we found hydrazine to be below detectable level (taken as 1/10 the minimum level of

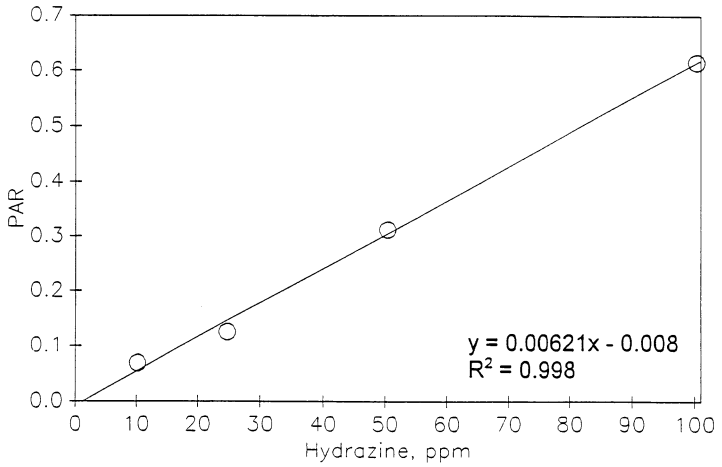

Fig. 4. Typical regression line for hydrazine (as benzalazine) in isoniazid syrup.

quantitation—or 1 ppm) in product stored at 0°C. The usual dosing regimen for isoniazid is 300 mg day^{-1} for 6 months. Assuming the product is stored at ambient temperature and decomposition continues linearly from the fourth through the sixth month, the concentration of hydrazine in the syrup at 6 months is projected to be ca. 18–19 ppm. These concentration levels are well in excess of the threshold level predicted by the Gaylor-Kodell extrapolation method referred to above. For example, on the twelfth day of treatment, the subject will receive a 30-ml dose of syrup containing minimally 1.6 ppm of hydrazine or 0.048 mg (Table 1). Assuming an average subject weight of 70 kg, the total body concentration will be 0.0007 mg kg^{-1} (ppm) or 700 ppt, almost 10 × higher than the threshold level predicted by the Gaylor-Kodell extrapolation method. Hydrazine concentrations at a later time in the dosing regimen will of course be much higher. The foregoing has assumed freshly prepared product. Commercial product, stored for a period of time at ambient

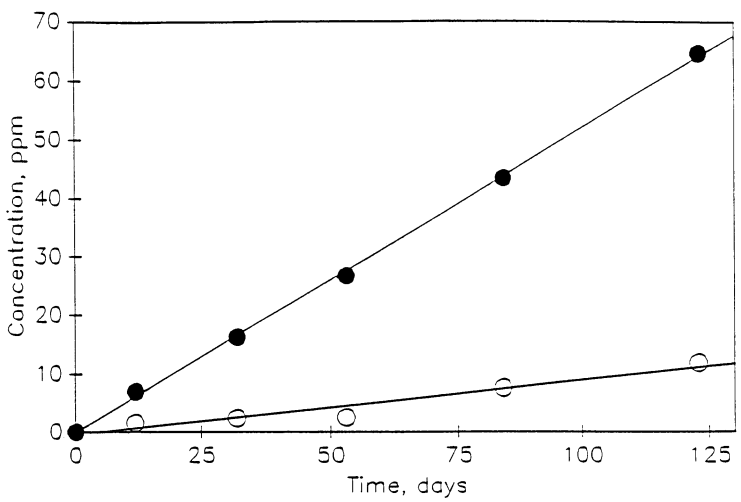

Fig. 5. Formation of hydrazine in simulated isoniazid syrup as a function of time when stored in glass bottles. ○: storage at ambient (23–27°C) temperature. ●: storage at 40°C. Formation of hydrazine at 0°C was below detectable level. The curves were the same for storage in glass or HDPE bottles.

temperature prior to dosing will contain considerably higher levels of hydrazine and, if stored at higher temperatures, higher levels yet.

Given the demonstrated decomposition of isoniazid to hydrazine in liquid formulations at ambient temperatures to produce levels that are predicted to increase tumor formation, we suggest that labeling be changed to require storage at sub-ambient temperatures as a first step toward a safer product. We will be working with the USP to revise the inadequacies in the current monograph.

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References

[1] USP 23, United States Pharmacopeial Convention, 1995, p. 846.
[2] Merck Index 11th ed., Merck, Rahway, NJ, 1989, pp. 4689, 5068.
[3] J. Juhász, J. Balo, G. Kendrey, Z. Krebsforsch. 62 (1957) 188–196.
[4] J.C. Arcos, Y.T. Woo, M.F. Argus, D.Y. Lai, Chemical Induction of Cancer, Academic Press, New York, 1982, p. 363.
[5] G.L. Mandell, M.A. Sande, in: A.G. Gillman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), The Pharmacological Basics of Therapeutics, 8th ed., Pergamon, New York, 1990, pp. 1146–1149.
[6] The Merck Manual of Diagnosis and Therapy, 16th ed., Merck, Rahway, NJ, 1992, pp. 139–140.
[7] F. Matsui, D.L. Robertson, E.G. Lovering, J. Pharm. Sci. 12 (1983) 948–951.
[8] C. Biancifiori, J. Natl. Cancer Inst. 44 (1970) 943–953.
[9] D.W. Gaylor, R.L. Kodell, J. Environ. Pathol. Toxicol. 4 (1980) 305–312.