determination of nitroglycerin in various dosage forms. The degradation products of nitroglycerin can be determined by a simple change of the mobile solvent system (methanol–water, 20:80) (Fig. 3). The assay is faster, more specific, and provides more stability-indicating information than the USP method.

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Hydrazine Levels in Formulations of Hydralazine, Isoniazid, and Phenelzine Over a 2-Year Period

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Abstract □ Hydrazine levels in formulations of hydralazine, isoniazid, and phenelzine have been measured over a 2-year period under ambient conditions and under temperature and humidity stress. Hydralazine tablets are stable under ambient conditions, but the hydrazine level in an injectable formulation increased from 4.5 to 10 μg/ml over a 23-month period. Isoniazid tablets are also stable, but hydrazine levels in an elixir and a pyridoxine combination product doubled to 44 μg/ml and 19 μg/tablet, respectively. Levels in phenelzine tablets appeared to remain constant at ~60 μg/tablet, with considerable tablet-to-tablet variation.

Keyphrases □ Hydrazine—levels in formulations of hydralazine, isoniazid, and phenelzine over a 2-year period □ Hydralazine—hydrazine levels in formulations over a 2-year period □ Isoniazid—hydrazine levels in formulations over a 2-year period □ Phenelzine—hydrazine levels in formulations over a 2-year period

Previous work demonstrated the presence of hydrazine in an isoniazid injectable product (1) and showed that isoniazid may hydrolyze to hydrazine (2, 3). Because hydrazine poses a risk of cancer in humans (4, 5), these observations prompted an assessment of other drugs derived from hydrazine that are available in Canada. These include carbidopa, hydralazine, isocarboxazid, and phenelzine, in addition to isoniazid. Hydrazine levels in isoniazid single-component tablet formulations were determined by TLC (2) and high-performance liquid chromatography (HPLC) (6) and by modifying a GLC procedure originally developed for the determination of phenelzine in urine (7). Methods for the determination of hydrazine in formulations of hydralazine (8), isoniazid elixir, isoniazid–pyridoxine combination tablets (8), and phenelzine (9) have also been developed. A 2-year normal and accelerated aging study of hydrazine formation in formulated products of hydralazine, isoniazid, and phenelzine has been completed and the results are reported in this paper.

Hydrazine is used in some syntheses of hydralazine (10), isoniazid (11), and phenelzine (12), and its presence in a formulated drug product may result from improper purification of the drug. All three drugs are known to degrade to hydrazine in solution (6, 9, 11), but there does not appear to be any published information on the formation of hydrazine in formulations of these drugs.

EXPERIMENTAL

Sample Preparation—Drug formulations were obtained directly from the manufacturer. All tablet samples were transferred to amber bottles for storage with at least five tablets in each bottle, and 1.0-ml aliquots of the elixir were transferred to culture tubes and securely capped. Phenelzine tablets from lot D were sealed in glass tubes (22 × 220 mm) by drawing out the top of the tube in a flame. The 100% relative humidity condition was achieved by sealing a 12-ml centrifuge tube containing 4 ml of water in with the tablets; tablets were not in direct contact with the water.

Storage Conditions—All formulations were aged under the temperature and humidity conditions given in Tables I, II, and III. Except as noted above, the humidity was controlled by placing silica gel (0% relative humidity) or an aqueous solution of sodium chloride (75 and 80% relative humidity) (13) in desiccators along with the product to be aged. The desiccators were placed in ovens[1] at the appropriate temperatures. Temperature variations were within 1.0° over the time of the experiments.

Procedure—All products were analyzed for hydrazine at the start of each study and at appropriate times thereafter. Assays were done in duplicate, usually on composites of five tablets, or composites of three ampules of an injectable or elixir or, for phenelzine, on single tablets. Hydrazine content (as the benzaldehyde derivative) in hydralazine (8), isoniazid–pyridoxine tablets, and isoniazid elixir (8) was determined by GLC using a 2% OV-101 column and a nitrogen–phosphorus detector. In phenelzine products and for some isoniazid analyses, hydrazine content (as the benzaldehyde derivative) was determined by HPLC (9) on a 5-μm silica gel column with a mobile phase of 6% chloroform in *n*-hexane and detection at 313 mm. In isoniazid single-component tablets after 2.5 and 6.8 months, hydrazine, as the acetone derivative, was determined by GLC on a 3% OV-225 column with a flame-ionization detector (7). The initial assessment of hydrazine in isoniazid tablets was made by TLC with

[1] Thelco Model 6 M and Freas Model 815, Precision Scientific.

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Table I—Hydrazine Levels in Hydralazine Products

Product	Time, months	Hydrazine Level, μg/tablet[a] RT, AH[b]	RT, 80%	37°, AH	37°, 80%
Tablets (10 mg)	0	Tr.[c]	Tr.	Tr.	Tr.
	7.5	0.03	0.22	0.03	0.06
	16	0.05	0.12	0.05	n.d.
	23	0.06	0.05	0.24	n.d.
Tablets (50 mg)	0	Tr.	Tr.	Tr.	Tr.
	7.5	n.d.	0.15	n.d.	0.8
	16	n.d.	n.d.	n.d.	1.3
	23	0.1	0.17	0.1	3.8
Injection (20 mg/ml;	0	4.5 μg/ml[d]	—	4.5 μg/ml[d]	—
hydrazine levels in	7.5	8	—	22	—
μg/ml)	16	12	—	76	—
	23	10	—	42[e]	—
Tablets (50 mg with	0	Tr.	Tr.	Tr.	Tr.
0.2-mg reserpine)	7.5	0.2	n.d.	n.d.	2.2
	16	n.d.	0.8	n.d.	3.2
	23	0.1	0.6	0.1	0.2
Tablets (25 mg with	0	Tr.	Tr.	Tr.	Tr.
0.1-mg reserpine and	7.5	n.d.	n.d.	n.d.	0.35
15-mg hydrochloro-	16	n.d.	n.d.	n.d.	0.35
thiazide)	23	0.05	0.05	0.07	0.15

[a] Minimum quantifiable amounts at 0, 7.5, 16, and 23 months were 0.001, 0.0005, 0.00025, and 0.000125%, respectively. Measurements were made with a Perkin-Elmer Model 3920 and Hewlett-Packard Models 5880 and 5840 gas chromatographs at 0, 7.5 and 16, and 23 months respectively. The amounts of drug injected on column were 10.0, 1.0, 2.0, and 4.0 μg, respectively. [b] RT = room temperature; AH = ambient humidity. [c] Tr. = trace, borders on the minimum detectable level (7×10^{-5} %). [d] This product is an aqueous solution. [e] Mean hydrazine level in five vials was 42 μg/ml, *RSD* 7.3%, range 42–57 μg/ml.

silica gel plates, run first in a solvent of methanol–chloroform (1:1) and then in acetone–methanol–glacial acetic acid (50:50:10) (2).

RESULTS AND DISCUSSION

Hydralazine—A preliminary examination of hydralazine products revealed traces of hydrazine in some tablet products and ~5 μg/ml in an injectable formulation. These results prompted a stability assessment of this drug. The products assessed, the test conditions, and the hydrazine levels at intervals over a 23-month period are presented in Table I. Over this period the sensitivity of the test method increased due to changes in the equipment available for the work and slight modifications made in the sampling procedure. Minimum quantifiable amounts are also given in Table I. Many of the results are close to the minimum quantifiable level of the method. This, coupled with the instability of hydrazine, probably accounts for the lack of a clear trend in hydrazine levels with time in some products.

The results show that there is no significant change in the hydrazine level in tablets stored under normal room conditions or at 37° and ambient humidity. At 80% relative humidity hydrazine levels increased in some products, more noticeably at 37°; but even at 37° not all tablet products were affected. At 37° and 80% relative humidity, the maximum level observed was ~3.8 μg in a 50-mg tablet after ~2 years. In the injectable product at room temperature and 37°, the hydrazine level increased from 4.5 to 12 and 76 μg/ml, respectively, in 16 months and then decreased, possibly due to decomposition of hydrazine rather than vial-to-vial variation in the hydrazine content. More rapid degradation to hydrazine in a liquid product would be expected. There is no indication that the presence of reserpine and/or hydrochlorothiazide had any effect on the formation of hydrazine from hydralazine.

Isoniazid—The isoniazid stability study was done in two parts. In the first, samples from two lots of tablets were stressed for 8 months under the conditions given in Table II. This work was undertaken before the analytical methodology was firmly established. The hydrazine assays were by TLC (initial), GLC (2.5 and 6.8 months), and HPLC (8.2 months). The GLC procedure (7) was modified to provide for the reaction of hydrazine with acetone to form an acetonide which was partitioned between water and ether and chromatographed at 45° on 3% OV-225 on Chromosorb WHP (100/120 mesh). The results (Table II) indicate only low levels of hydrazine contamination in products kept at room temperature and 0% relative humidity. Hydrazine formation is promoted at higher humidity, as would be anticipated, but the effect is not pronounced except in one product at 60° and 75% relative humidity.

In the second part of the work, an isoniazid–pyridoxine combination product and an isoniazid elixir were evaluated (Table II). The hydrazine level in tablets at ambient humidity (room temperature and 37°) rose to a constant value of ~20 μg/tablet. At 80% relative humidity, hydrazine levels were much higher, but again there was some indication of the attainment of a steady-state hydrazine level. In the elixir at room temperature, the hydrazine level increased to a steady level of ~40 μg/ml, but at 37° it declined to a steady level of 2–4 μg/ml. A temperature-dependent steady-state level of hydrazine would result when the rate of formation of hydrazine and its rate of decomposition are in balance.

Phenelzine—The stability study of phenelzine tablets began when samples from lot C were tested under the temperature and humidity conditions given in Table III. After 4 months it was observed that the cores of individual tablets varied in color from white to beige. Single tablets were analyzed for hydrazine, and it was found that hydrazine levels increased roughly with the color of the tablet, with a range of 34–82 μg/tablet. The data obtained from the sample stored at 37° and 80% relative humidity indicated a loss of hydrazine due to volatilization and/or chemical breakdown. In view of these uncertainties, a second lot (D) of tablets were obtained, repackaged in sealed containers, and tested to find out what became of the hydrazine (Table III).

The first observation that can be made from the data is that hydrazine levels decreased during storage at 80 and 100% relative humidity. The levels decreased sharply at 100% relative humidity and the tablets showed extensive deterioration. Hydrazine levels also decreased at 80% relative humidity and 37°. The data obtained from the samples stored in sealed containers show that these decreases can be attributed to chemical degradation rather than evaporation of the hydrazine. At ambient humidity, both at room temperature and 37°, there may have been some increase in the hydrazine content to a steady level, but once this state was reached (after a few months) there did not appear to be any further increase.

Table II—Hydrazine Levels in Isoniazid Products

Product	Time, months	Hydrazine Level, μg/tablet RT, 0%	RT, 75%	45°, 0%	45°, 75%	60°, 0%	60°, 75%
Tablets (100 mg)	0[a]	Tr.	Tr.	Tr.	Tr.	Tr.	Tr.
(Lot A)	2.5	<2	2	—[c]	5	1	—
	6.8	2	3	1	6	2	47
	8.2	1[b]	3.6[b]	1.5[b]	7[b]	3[b]	70[b]
Tablets (100 mg)	0[a]	n.d.[d]	n.d.	n.d.	n.d.	n.d.	n.d.
(Lot B)	2.5	—	—	—	—	—	—
	8.2	2.4[b]	2.5[b]	3.4[b]	6[b]	4.4[b]	6[b]
		RT, AH	RT, 80%	37°, AH	37°, 80%		
Tablets (300 mg	0	8	8	8	8		
with 15 mg	7.5	12	58	15	201		
pyridoxine HCl)	16	n.d.	96	21	257		
	23	19	112	22	189		
Elixir (10 mg/ml)	0	19.5 μg/ml	—	19.5 μg/ml	—		
	7.5	36	—	4	—		
	16	43	—	5	—		
	23	44	—	2	—		

[a] *Determinations at time zero were by* TLC. The minimum quantifiable level was 0.006%. Trace is ~0.003%. [b] Determination by HPLC. The minimum quantifiable amount was 0.0002%. [c] Not determined. [d] None detected.

Table III—Hydrazine Levels in Phenelzine Products

Product	Time, months	Hydrazine Level, μg/tablet[a] RT, AH Mean (CV)[a]	RT, AH Range (n)[b]	RT, 80% Mean (CV)	RT, 80% Range (n)	37°, AH Mean (CV)	37°, AH Range (n)	37°, 80% Mean (CV)	37°, 80% Range (n)
Tablets (15 mg) (Lot C)	0	47[c]	—[d]	47[c]	—	47[c]	—	47[c]	—
	4	51(44)	34–82 (4)	37[c]	—	—	—	23[c]	—
	13	79(36)	43–110(5)	40(37)	27–59(5)	—	—	5.5(40)	1.8–9.1(12)
	19	72(57)	43–135(5)	—	—	—	—	—	—
	24	63(49)	23–128(20	—	—	—	—	—	—
Tablets (15 mg) (Lot D)	0	40(28)	28–64 (30)	40(28)	28–64(30)	40(28)	28–64 (30)	40(28)	28–64(30)
	4	63(46)	34–115(14)	2.7(45)[e]	1.5–4.8(5)[e]	58(35)	38–82 (5)	n.d.[e,f]	n.d.[e,f]
	11	47(29)	33–68 (10)	1.3[e,g]	—	61(40)	35–101(10)	—	—
	16	56(37)	30–86 (20)	—	—	68(45)	38–122(10)	—	—

[a] Coefficient of variation. [b] Number of tablets assayed. [c] Composite of 10 tablets. [d] — Not determined. [e] 100% relative humidity. [f] n.d. = none detected. [g] Composite of 15 tablets.

Table IV—Distribution of Hydrazine[a] in Phenelzine Tablets

Time of Assay, Months[b]	Number of Tablets Assayed	Percentage of Tablets in each Range of Hydrazine Level <30 μg	30–45 μg	45–60 μg	60–75 μg	75–90 μg	90–105 μg	>105 μg
	Lot C							
5	4	—	50	25	—	25	—	—
13	5	—	20	—	40	—	20	20
19	5	—	60	—	—	—	20	20
24	20	5	40	20	5	5	10	15
	Lot D							
0	30	3	74	10	13	—	—	—
4	14	—	43	14	7	7	22	7
11	10	—	50	30	20	—	—	—
16	20	—	45	10	15	30	—	—

[a] Tablets stored at ambient temperature and humidity. [b] Time elapsed since tablets were assayed at the start of the study.

The intertablet variation in hydrazine level is presented in Table IV. The variation appeared to be present in the tablets as they were obtained from the manufacturer, and the data do not support the view that there were significant changes in hydrazine levels as the study progressed. The tablet-to-tablet variation suggests that hydrazine is formed during manufacture under local reaction conditions, but specific conditions are unknown.

The work described herein was done to identify major stability problems with respect to increases in the hydrazine levels with time in the drugs examined. Other facets of the stability of these products, such as degradation of the drug itself, were not included in this work. Except for samples stored at ambient temperature and humidity, samples were repacked so that they would be fully exposed to the conditions of the experiment. This was done because the objective of the work was to examine the behavior of the formulated products, not the ability of the packaging to protect the product from the environment.

The results show that hydrazine itself decomposes in some formulations, *e.g.*, the isoniazid elixir at 37° (Table II) and phenelzine tablets at 37° and 80% relative humidity (Table III). Some data, like that for isoniazid–pyridoxine tablets indicate the hydrazine concentration increases to a fixed level and remains constant. This and the results at two temperatures for the isoniazid elixir suggest that hydrazine levels in some products are steady-state levels. The actual value of the steady-state level would depend on the nature of the formulation excipients, the rates of hydrazine formation and decomposition, and the relative effect of temperature on these rates.

Hydrazine is a metabolite of isoniazid (14–16) and hydralazine (17) in humans. It may also be a metabolite of phenelzine[2]. Any assessment of hydrazine contamination in formulated products should include an evaluation of the metabolic data.

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