Determination of Hydrazine in Pharmaceuticals IV: Hydrazine and Benzylhydrazine in Isocarboxazid

E. G. LOVERING[x], F. MATSUI, D. ROBERTSON, AND N. M. CURRAN

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Abstract □ A GC procedure for the simultaneous determination of hydrazine and benzylhydrazine in isocarboxazid raw material and tablet formulations has been developed. The method is based on the reaction of benzoyltrifluoroacetone with hydrazine and benzylhydrazine to form the corresponding pyrazole derivatives. The minimum detectable amounts of hydrazine and benzylhydrazine in isocarboxazid are 0.002 and 0.02%, respectively.

Previous papers in this series described methods for the determination of hydrazine in isoniazid,[1] phenelzine,[2] and hydralazine[3] and reported the amounts typically found in commercial formulations. This work has now been extended to isocarboxazid, a monoamine oxidase inhibitor, which could be contaminated with hydrazine and benzylhydrazine as a carry over from the synthesis[4] or by chemical degradation of the drug. Hydrazine is a mutagen[5-7] and a carcinogen in laboratory animals.[8] Concern over its presence in drug products led to a proposal for regulatory action in the United States[9] and to the recall of an isoniazid product in Canada.[10]

In previous work,[1-3] hydrazine was determined as its benzaldehyde derivative, benzalazine. However, with phenalkyl-substituted hydrazines such as benzylhydrazine and phenethylhydrazine, the hydrazones formed with benzaldehyde were found to degrade. Thus it was necessary to develop a different procedure for the impurities in isocarboxazid. Benzoyltrifluoroacetone (BTA) was found to be a satisfactory derivatizing agent because both the hydrazine and benzylhydrazine derivatives are stable and are chromatographically resolved (Fig. 1).

Experimental Section

Materials—Hydrazine dihydrochloride and benzoyltrifluoroacetone from Sigma Chemical Co., St. Louis, MO; benzylhydrazine dihydrochloride from Pfaltz and Bauer Inc., Stanford, CT; 5-chloro-2-(methylamino)benzophenone from Aldrich Chemical Co., Milwaukee, WI; and isocarboxazid from Hoffmann LaRoche Ltd., Vaudreuil, P.Q., Canada, were used as received. Solvents and reagents were commercial analytical grade, except the chloroform and *n*-heptane from Caledon Laboratories Ltd., Georgetown, Ont., Canada, which were glass distilled.

A Hewlett-Packard Model 5880A gas chromatograph equipped with a nitrogen–phosphorous detector and a coiled glass column (0.91 m × 4 mm i.d.) packed with 2% OV-101, from Pierce Chemical Co., Rockford, IL, on Chromosorb G-HP from Johns Manville, Denver, CO., (80/100 mesh) was used. Instrument temperatures were: injector port, 275°C; column, 220°C; detector, 300°C. Gas flows were: argon, 40 mL/min; air, 50 mL/min; hydrogen, 3 mL/min. A solution of benzoyltrifluoroacetone (4 mg/mL) in methanol:water (1:1) was prepared by dissolving it in methanol and then diluting to volume with water. The internal standard was a solution of 5-chloro-2-(methylamino)benzophenone in *n*-heptane (7.5 μg/mL), prepared fresh daily.

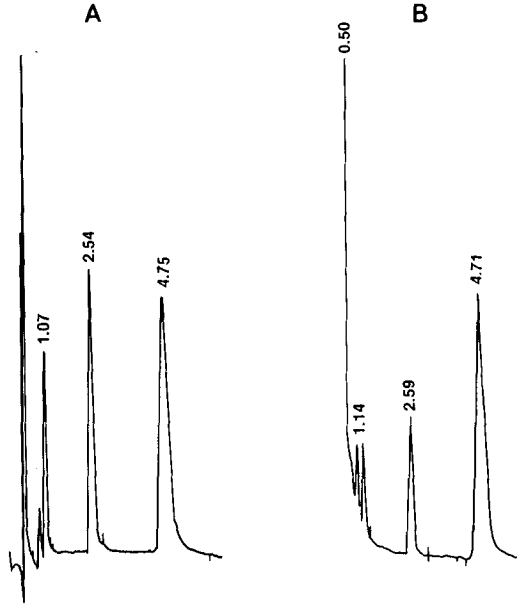

Figure 1—*Chromatograms showing the resolution of hydrazine and benzylhydrazine as their benzoyltrifluoroacetone derivatives and of 5-chloro-2-(methylamino)benzophenone, the internal standard. Key: (A) standard solution. The on-column amounts are equivalent to 152 pg hydrazine (1.07 min), 1608 pg benzylhydrazine (2.54 min), and 7160 pg 5-chloro-2-(methylamino)benzophenone (4.75 min). (B) Aqueous solution of isocarboxazid raw material after 12 months storage at 37 C°. The on-column sample is equivalent to 10.66 ng isocarboxazid and the amounts on column are equivalent to 82.6 pg hydrazine (1.14 min), 1052 pg benzylhydrazine (2.59 min), and 7479 pg 5-chloro-2-(methylamino)benzophenone (4.71 min).*

Detector Sensitivity—Over a working day the sensitivity of a nitrogen–phosphorous detector may change. Thus, the frequent injection of standards in close proximity to the sample is necessary to avoid error in the analysis. As a guide to setting the detector voltage, a solution containing the benzoyltrifluoroacetone derivative equivalent to 0.150 ng of benzylhydrazine base should give a peak height of 5–7 mm. The sensitivity of the procedure can be increased by a factor of at least five by using a higher element voltage, but this will shorten the detector lifetime, and the extra sensitivity is not necessary for the purpose of this work.

Standard Curve—A solution containing hydrazine dihydrochloride (200 μg/mL, equivalent to 61.04 μg/mL of hydrazine) and benzylhydrazine dihydrochloride (800 μg/mL, equivalent to 500.9 μg/mL of benzylhydrazine) in 0.1 M HCl was prepared. The solution was diluted with 0.1 M HCl to obtain four standard solutions containing approximately 0.30, 0.45, 0.75, and 1.50 μg/mL of hydrazine and 2.50, 3.75, 6.25, and 12.5 μg/mL of benzylhydrazine.

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A 5.0-mL aliquot of each solution was transferred into separate 150 × 20-mm culture tubes fitted with Teflon-lined screw caps; 5.0 mL of chloroform was added to each tube and the tubes were rotated at 30 rpm for 30 min. A 2.0-mL aliquot of the aqueous layer from each tube was placed into separate 150 × 20-mm culture tubes containing 1.0 mL of the derivatizing reagent solution. The tubes were heated on a dry bath at 65°C for 30 min and then cooled to room temperature. To each tube was added 15.0 mL of internal standard solution, and the tubes were again tumbled at 30 rpm for 30 min. Duplicate 1-μL aliquots of the upper phase were chromatographed and the slopes of the hydrazine and benzylhydrazine curves were calculated from the ratios of the heights of the appropriate pyrazole derivative peaks to the heights of the internal standard peaks versus the corresponding weight ratios.

Day-to-day Calibration—Two standard solutions of hydrazine dihydrochloride and benzylhydrazine dihydrochloride were prepared each day. The usual respective concentrations as hydrazine and benzylhydrazine were ~0.5 and 3.75 μg/mL, and 0.75 and 6.25 μg/mL. A 5.0-mL aliquot of each standard was treated as described above, and 1-μL injections were chromatographed at regular intervals during the day.

Drug Raw Material and Tablets—Accurately weighed isocarboxazid drug raw material, or a powdered tablet composite equivalent to 20 mg of drug, was tumbled with 5.0 mL of 0.1 M HCl for 30 min in a culture tube fitted with a Teflon-lined screw cap; 5.0 mL of chloroform was then added, and the sample was again tumbled for 30 minutes. After centrifugation, a 2.0-mL aliquot of the upper aqueous layer was derivatized as described under standard curve preparation, and 1-μL aliquots of the organic phase containing the internal standard were injected.

Quantitation was based on the ratio of the hydrazine:benzoyltrifluoroacetone or benzylhydrazine:benzoyltrifluoroacetone peak height to that of the internal standard peak height. Calibration standards were prepared to approximate the levels of hydrazine and benzylhydrazine in the drug raw materials and formulations being analyzed.

Results and Discussion

The method is based on the reactions of hydrazine and benzylhydrazine with benzoyltrifluoroacetone to form the corresponding pyrazole derivatives[11,12] (Scheme I) which are subsequently separated and quantitated by GC. The MS of the derivatives gave m/z values of 212 and 302 for the hydrazine and benzylhydrazine derivatives, respectively, indicating the possible condensation of 1 mol of hydrazine with 1 mol of benzoyltrifluoroacetone, followed by an intramolecular condensation, and resulting in total elimination of 2 mol of water. The pyrazole derivatives of hydrazine and benzylhydrazine elute at 1.1 and 2.6 min, respectively, and the internal standard elutes at 4.7 min.

The volume of 0.1 M HCl required to maximize extraction of the hydrazine and benzylhydrazine from the tablet matrices was determined by shaking portions of powdered tablet composites equivalent to 10, 20, and 30 mg of isocarboxazid with 5.0 mL of solvent for 30 min. No benzylhydrazine was observed, but hydrazine was detected (minimum detectable level of 0.002%) when the ratios were 20 and 30 mg of isocarboxazid to 5.0 mL of 0.1 M HCl. At least 40 mg of isocarboxazid drug raw material was soluble in 5 mL of 0.1 M HCl.

The acidic tablet extract or drug solution was shaken with chloroform to remove isocarboxazid prior to derivatization and chromatography. Removal of the drug was necessary to obtain a noise-free chromatographic baseline; additionally, it precluded the possibility of an overestimation of the impurities due to breakdown of the drug during derivatization, although it was shown that breakdown does not, in fact, occur during the analysis. No peaks were observed in the chromatogram of the reagent blank. When isocarboxazid was subjected to the entire procedure, but without the addition of benzoyltrifluoroacetone, no peaks were contributed to the chromatogram by the drug or any impurities in it. Also, the chromatograms were virtually identical whether 5.0, 10.0, or 20.0 mL of chloroform was used. The chloroform extraction step was shown not to remove detectable amounts of hydrazine or benzylhydrazine by the comparison of the peak height ratios of the standard solutions which were derivatized without the chloroform wash and the peak height ratios of a drug raw material and a tablet composite spiked with the same standard solutions. The chloroform phase was separated and back-extracted with 2.0 mL of 0.1 M HCl, and the assay was repeated. No hydrazine or benzylhydrazine was detected.

The optimum derivatization temperature was determined by carrying out the reaction on a solution containing 0.978 μg/mL hydrazine and 11.30 μg/mL benzylhydrazine for 30 min at 55°C, 60°C, 65°C, and 70°C. For hydrazine, the slope values of 38.36, 37.61, 38.31 and 38.58 obtained at the above temperatures, respectively, indicated the reactions to have reached completion at 55°C. However, for benzylhydrazine, the slope values of 4.55, 4.72, 4.82, and 4.82 obtained at the above temperatures, respectively, indicated that a temperature of at least 60°C was required to attain similar status. Thus, a derivatization temper-

Derivatization Reactions

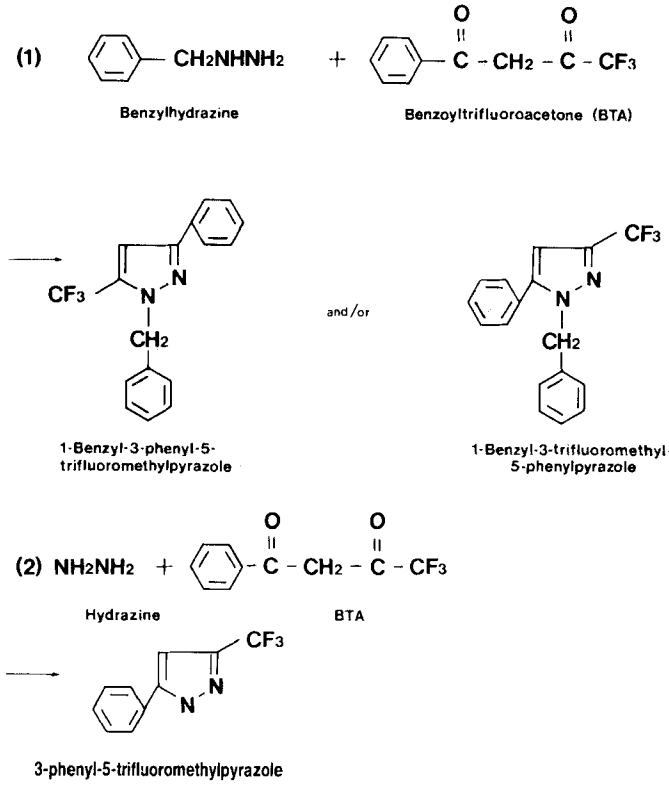

Table I—Hydrazine and Benzylhydrazine in Isocarboxazid Drug Raw Materials and Tablet Formulations

Product	Lot No.	Hydrazine, %	Benzylhydrazine, %
Drug raw material	A	ND[a]	ND
	B	ND	ND
	C[b]	0.008[c]	ND
Tablet, 10 mg	D	trace[d]	ND
Tablet, 10 mg	E	trace	ND
Aqueous solution[e]		0.775	9.87

[a] None detected. [b] Sample ~15 years old. [c] Mean of 5 determinations; RSD, 13.0%. [d] Minimum amount detectable, ~0.002%. [e] Aqueous solution of drug raw material, lot B.

ature of 65°C was selected to ensure complete derivatization of both compounds.

The time required for benzoyltrifluoroacetone to react quantitatively with these compounds was determined by allowing the reaction to proceed at 65°C for various periods of time. Increases in the peak height ratios were observed for reaction times ≤20 min, after which no change was observed to 45 min for either compound. At the same time, these findings indicated that the benzoyltrifluoroacetone derivatives of both the hydrazine and benzylhydrazine were stable for at least 45 min. This was further substantiated when aliquots of a tablet extract derivatized for 20, 30, and 45 min exhibited virtually identical chromatograms (0.002% hydrazine and no detectable benzylhydrazine).

The effect of benzoyltrifluoroacetone concentrations of 2, 4, and 6 mg/mL on the completeness of derivatization was also investigated. These levels were selected to provide 1:1 molar ratios corresponding to 25, 50, and 75% breakdown of isocarboxazid. No significant differences were observed. A concentration of 4 mg/mL of benzoyltrifluoroacetone was selected for use in the method.

To demonstrate that the benzoyltrifluoroacetone derivatives of hydrazine and benzylhydrazine were adequately extracted from the 0.1 M HCl layer of a powdered tablet extract spiked with these impurities, the aqueous phase after derivatization was shaken with *n*-heptane in the ratios of 2:10, 2:15, and 2:20. The respective slope values were 39.44, 38.81, and 39.59 for hydrazine and 4.71, 4.62, and 4.62 for benzylhydrazine. The results indicated that the partition was constant over the range investigated. The method calls for an aqueous to organic volume ratio of 2:15.

Standard curves over the range 35–300 pg of hydrazine and 375–1600 pg of benzylhydrazine, both injected as the benzoyltrifluoroacetone derivatives, were linear with intercepts not significantly different from zero. Mean slopes of 37.39 with a relative standard deviation of 4.6% and 4.67 with a relative standard deviation of 2.4% were obtained for hydrazine:benzoyltrifluoroacetone and benzylhydrazine:benzoyltrifluoroacetone, respectively. This range represents hydrazine levels of 0.006–0.056% and benzylhydrazine levels of 0.070–0.30% for injections which correspond to 533 ng of isocarboxazid. The drug, however, is not present since it was removed by the chloroform extraction step.

The reproducibility of the chromatographic system was established by chromatographing six aliquots each of two different concentrations of the BTA derivatives. The relative standard deviations of the respective slopes were 1.27 and 1.05%, for on-column amounts equivalent to 101.3 and 76.00 pg of hydrazine, and 0.64 and 1.51% for on-column amounts equivalent to 1001.3 and 804.2 pg of benzylhydrazine. It was possible to establish the reproducibility of the method only with respect to the determination of hydrazine because no benzylhydrazine was detected in any drug sample investigated. A relative standard deviation of 13.0% was obtained when an old lot of the drug raw material was analyzed five times (Table I). Absolute accuracy of the method as applied to table analysis could not be reliably estimated because of the difficulty involved in the preparation of an exact duplicate of the sample. Thus, it was only possible to establish the recoveries of hydrazine and benzylhydrazine from formulations to which 0.1 M HCl solution, containing known amounts of these substances at the extraction step, was added. Comparison of these recoveries to those obtained with the standard solutions alone taken through the procedure indicated that the recovery ranged between 96.7–99.6% for hydrazine and 93.1–96.5% for benzylhydrazine. The minimum detectable amounts of hydrazine and benzylhydrazine as the derivatives were ~12 pg and 108 pg, respectively. The minimum reliable quantifiable levels were ~35 pg and 350 pg, respectively. This allowed for the detection of hydrazine at levels of ~0.002% and of benzylhydrazine at ~0.020% in drug products.

Three lots of drug raw materials and two lots of a tablet formulation were examined (Table I). No benzylhydrazine was detected in any sample investigated. No hydrazine was detected in two recently acquired lots of drug raw materials. An old lot of the raw material contained 0.008% hydrazine and the two tablet lots contained trace amounts (~0.002%) of hydrazine.

An aqueous solution of drug raw material was stored at 37°C for 1 year. At the end of this time, it contained 0.775% hydrazine and 9.87% benzylhydrazine. These data indicate that the drug in acidic solution at 37°C decomposes to hydrazine and benzylhydrazine. No hydrazine or benzylhydrazine was detected in tablets which were stored for 7 months at 37°C and 80% relative humidity.

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