Assay of Nitrofurantoin Oral Suspensions Contaminated with 3-(5-Nitrofurfurylideneamino)hydantoic Acid

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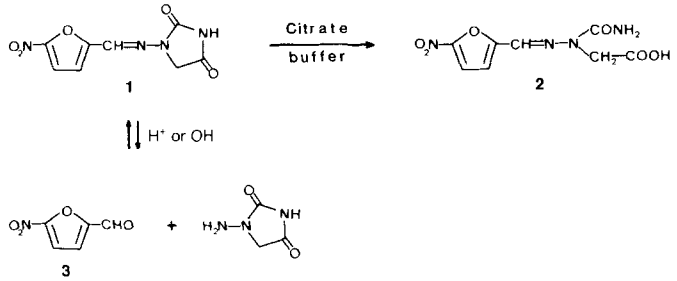
Abstract □ A reversed-phase high-performance liquid chromatographic (HPLC) analysis of oral suspensions for nitrofurantoin (**1**) and 3-(5-nitrofurfurylideneamino)hydantoic acid (**2**), an impurity derived from **1**, is presented. The concentration of the impurity ranged from 20 to 300 μg/mL in several lots of commercial oral suspensions. Conversion of **1** to **2** with citrate buffer, an excipient in the oral suspension, was achieved; selective hydantoin ring cleavage was accomplished in preference to the generally observed cleavage at the azomethine linkage. The hydantoic acid **2** was synthesized and identified by NMR, IR, TLC, and elemental analysis.

A nitrofurantoin (**1**) oral suspension, examined by HPLC, was found to contain an impurity generated by the reaction of the drug with citrate buffer, an excipient. The impurity was identified as 3-(5-nitrofurfurylideneamino)hydantoic acid (**2**) by comparison with synthetic material. An efficient synthesis of the hydantoic acid **2** as well as HPLC and TLC analyses of **1** and **2** are described here.

Hydrolysis is a major source of drug decomposition in formulations, and pH buffering can lead to stability problems.[1] As shown in Scheme I, hydrolysis of **1** at the azomethine chain[2,3] may reversibly generate 5-nitro-2-furaldehyde (**3**) and 1-aminohydantoin, whereas hydrolysis at the hydantoin ring can produce the hydantoic acid **2**; literature reports of the latter hydrolysis were not found. However, the selective heterocyclic ring cleavage of **1** to yield **2** in citrate buffer was observed and appears to explain the presence of **2** in buffered suspensions of the drug. The hydantoic acid **2** may also appear as a side product in the synthesis of **1**.[4]



Scheme I—Nitrofurantoin cleavage at the azomethine chain or at the hydantoin ring.

In an effort to improve the current compendial methods,[5] HPLC assays for **1** and possible contaminants (e.g., nitrofurazone) in dosage forms were investigated. HPLC assays for **1**[6] and two of its metabolites[7] in plasma and urine were tried but found unsuitable. In the former method, **1** was not separated from nitrofurazone, and in the latter, nitrofurantoin had a retention time of >1 h. During development of an HPLC method suitable for compendial use, **2** was found in the commercial suspension. This paper reports HPLC assays for **1** in the presence of **2** and for **2** itself.

Experimental Section

Materials—Reagent-grade solvents and chemicals were used unless otherwise described. Nitrofurantoin (**1**) from Norwich Eaton Pharmaceuticals, Inc., Norwich, NY, was assayed before use (99.8% by UV), and nitrofurazone (**4**) was a USP reference standard (Rockville, MD). Ethyl hydrazinoacetate hydrochloride (Pfaltz and Bauer, Inc.), 5-nitro-2-furaldehyde (99% from Aldrich Chemical Co.), and acetanilide (Matheson, Coleman and Bell, Gibbstown, N.J.) were used as received. Deionized Milli-Q water (Millipore Corp., Bedford, MA) was used to prepare mobile phases for HPLC, to purify the ion-exchange resin, and for reactions where indicated. The strong-acid cation-exchange resin (Duolite C-20H, Diamond Shamrock, Cleveland, OH) was a high-capacity, polystyrene nuclear sulfonic acid resin in the hydrogen form and was purified before use by column treatment.[8,9]

Instruments—An SP3-200 Pye Unicam grating IR spectrophotometer (KBr pellets) and a T60A Varian 60-MHz NMR spectrometer were used. All melting-point measurements were made on a Fisher-Johns melting-point apparatus. TLC sheets (Silica gel 60 F-254 on aluminum support, E.M. Laboratories, Elmsford, NY), precoated with silica gel, were developed in chloroform:methanol:ammonium hydroxide (6:4:0.5).

A Waters Associates 6000A pump, a WISP 710B injector, and a 450 UV detector were used for the assays. Another HPLC (Hewlett-Packard 1084A) with a UV-visible variable-wavelength detector (model 79875A) was used for qualitative spectral examination of eluted materials. A 300- × 3.9-mm μ-Bondapak C_{18} reversed-phase column (10-μm particle size, Waters Associates) with detection at 254 nm was used to assay for **1**, and a 250- × 4.6-mm Hibar-II LiChrosorb RP-18 (10-μm particle size, EM Laboratories, Cincinnati, OH) reversed-phase column with detection at 375 nm was used to assay for **2**. The flow rate for both procedures was ~1.5 mL/min.

A pH 7.0 phosphate buffer was prepared. Monobasic potassium phosphate (6.8 g) was dissolved in ~500 mL of water. Sodium hydroxide solution (1.0 M, 30.0 mL) was added, the mixture was diluted to exactly 1 L with water, and the pH was checked. Acetonitrile (120 mL) was diluted to 1 L with the pH 7.0 buffer, and the mixture was filtered, deaerated, and used as the mobile phase.

Acetanilide (~13 mg) was dissolved in 200 mL of the mobile phase and was used as the internal standard solution. About 25 mg of **1** was weighed accurately and transferred to a 100-mL volumetric flask with ~50 mL of dimethylformamide. Water (20 mL) was added, and the mixture was cooled to room temperature and diluted to volume with dimethylformamide. A 4.0-mL aliquot of this solution was mixed with 15.0 mL of internal standard solution to make Standard I. For Standard II, a solution (125 μg/mL) of **2** was accurately prepared in methanol. A 2.0-mL aliquot of this solution was diluted to 100.0 mL with methanol.

Sample Preparation—*Sample I*—To a 5-mL aliquot of suspension in a 100-mL volumetric flask were added 20 mL of water and 50 mL of dimethylformamide, and the flask was shaken for 10 min. The mixture was cooled to room temperature and diluted to volume with dimethylformamide. A portion of the solution was centrifuged, and a 4.0-mL aliquot of the supernatant was mixed with the internal standard solution (15.0 mL). Five milliliters of the mixture was filtered through a 5.0-μm Type LS filter (Millipore Corp.), and the first 2 mL of the filtrate was discarded.

Sample II—A 5-mL aliquot of suspension was transferred to a 100-mL volumetric flask with 70 mL of methanol. The mixture was shaken for 5 min, diluted to volume with methanol, and mixed. A portion of the solution was centrifuged. Five milliliters of the supernatant was filtered through a 5.0-μm LS filter, and the first 2 mL of filtrate was discarded.

Procedure I—Fifteen microliters of Standard I and Sample I were injected into the liquid chromatograph. The operating conditions were adjusted so that the retention time of the peak for **1** was ~8 min and its height was about 0.5 full scale. The ratio of the peak area of **1** to that of the internal standard was obtained with Standard I and Sample I and was used to calculate the amount of the nitrofurantoin in the suspension.

Procedure II—Thirty microliters of Standard II and Sample II were injected into the liquid chromatograph. The operating conditions were adjusted so that the retention time of the peak for **2** was ~3–6 min and its height was about 0.1 full scale. The peak heights of **2** were measured with Standard II and Sample II and were used to calculate the amount of **2** in the suspension.

Sodium Salt of 3-(5-Nitrofurfurylideneamino)hydantoic Acid (2)—A mixture of 2.5 g (0.01 mol) of **1** monohydrate, 8.8 g (0.03 mol) of sodium citrate dihydrate, 2.0 g (0.01 mol) of citric acid, and 2.41 g (0.01 mol) of sodium saccharin dihydrate in 500 mL of deionized water was refluxed with stirring. The color of the solution was orange after 15 min and black after 13 h, at which time the heating was discontinued. The mixture was allowed to stand ~24 h at room temperature, and then the solid material was removed by filtration. Aliquots of the mixture were taken during the heating period, treated to remove **1**, as above, and examined by HPLC and TLC for **2** content and for comparison with the impurity **2** in a commercial oral suspension. The filtrate was saturated with sodium chloride and extracted twice with 375-mL portions of 2-butanone. The organic phase was concentrated to ~200 mL and allowed to stand at least 24 h. The precipitated material was washed three times with cold 2-butanone, filtered, and dried to give 400 mg of the sodium salt of **2** as a yellow solid, mp > 300°C; IR (KBr): 1630 cm^{-1} (carboxylate). Recrystallization from 2-butanone gave an analytical specimen which showed one spot on TLC and one peak on HPLC.

Anal.—Calc. for $C_8H_7N_4O_6Na$: C, 34.55; H, 2.54; N, 20.14. Found: C, 34.84; H, 2.78; N, 20.07.

Isolation of 2 as the Free Acid—To 100 mg of the sodium salt of **2** was added 4 mL of distilled water. The mixture was filtered, and hydrochloric acid (3 M) was added to the filtrate until the solution was acidic (pH ~2). The solid material was removed by filtration, washed with water, and dried to give 65 mg of **2** as a yellow solid: mp 238–239°C (dec.) [lit.[4] mp 234°C (dec.); mp (authentic specimen Norwich Eaton Pharmaceuticals) 239–241°C (dec.); and mixture mp with the authentic sample 238–239°C (dec.)]; IR (KBr): 3500–3100 (OH and NH), 3470 (OH or NH), 3280 (NH), 3328 (NH), 1715 (CO_2H), and 1650 (amide) cm^{-1}; ^1H NMR (Me_2SO-d_6): δ 4.72 (s, 2, CH_2), 7.42 (d, 1, J = 4 Hz, H_3), 7.80 (d, 1, J = 4 Hz, H_4), 7.68 (s, 1, CH—N), and 7.03 ppm (s, 2, $CONH_2$); TLC: R_f 0.6.

Anal.—Calc. for $C_8H_8N_4O_6$: C, 37.51; H, 3.15; N, 21.87. Found: C, 37.38; H, 3.17; N, 21.68.

3-(5-Nitrofurfurylideneamino)hydantoic Acid (2)—Previously described methods[4,10] were modified. A mixture of ethyl hydrazinoacetate hydrochloride (1.54 g, 0.01 mol), 0.50 g of dry purified cation-exchange resin, and 11.6 mL of deionized water were refluxed (magnetic stirring) for 3 h. The mixture was filtered, the filtrate was diluted to 20 mL with deionized water, and sodium bicarbonate (0.84 g) was added until the solution was pH 8. Nitrogen gas was bubbled through the solution for 5 min, and then potassium cyanate (1 g, 0.0123 mol) was added over 1 h with continuous stirring. 5-Nitro-2-furfuraldoxime (0.78 g, 0.005 mol, prepared from 5-nitro-2-furfuraldehyde[11]) and sulfuric acid (2.5 mL, 20%) were added, and the mixture was refluxed with stirring for 15 min. The dark-colored mixture was allowed to stand overnight and then was filtered to remove the dark-colored insoluble solids (0.34 g). The solvent was removed slowly under reduced pressure (caution, hood required) to give, after removal of the solid by filtration, 300 mg (23% yield) of **2** as a yellow solid. The material was dissolved in aqueous sodium carbonate solution and reprecipitated with dilute acid[4] to give material [mp 235–238°C dec; mixture mp (with **2** obtained by citrate buffer hydrolysis of **1**) 236–237°C (dec.)]. This material was identical (TLC and IR) with the authentic sample (Norwich Eaton) of **2** and with **2** obtained by citrate buffer hydrolysis of **1**.

Results and Discussion

The interconversion of hydantoic acids and hydantoin (Scheme I) is discussed in a review.[12] Ring closure is favored by dilute acid and *N*-substitution.[12,13] Long treatment with dilute base or acid completely disrupts the uride system and provides an important method for synthesis of α-amino acids.[12,14–16] Nevertheless, the present study shows that prolonged treament of the *N*-substituted hydantoin **1** with acidic citrate buffer results in a convenient one-step synthesis of **2** from the commercially available **1**.

The hydantoic acid impurity **2** was first detected in the oral suspension of **1** by HPLC (Fig. 1A) and, via stop-flow scan of the eluate, was shown to have a UV spectrum almost identical to that of **1**. Nitrofurazone, a common impurity of **1**, is also separated by the TLC and HPLC systems used.

A reaction of **1** in a mixture of common drug excipients (citric acid, sodium citrate, and sodium saccharin) resulted in

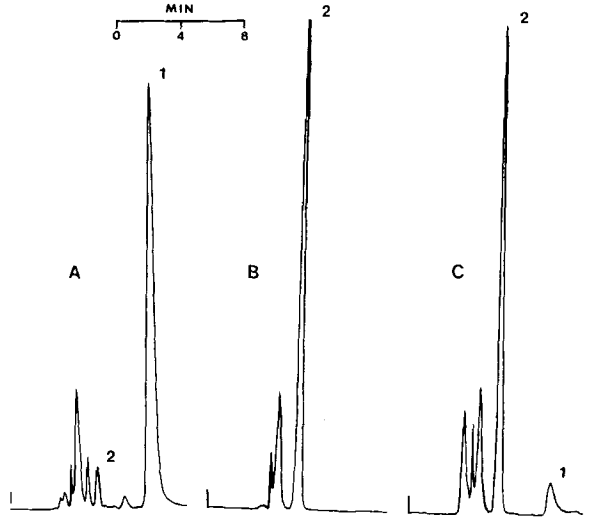


Figure 1—*Reversed-phase high-performance liquid chromatograms. Key: (1), nitrofurantoin; (2), 3-(5-nitrofurfurylideneamino)hydantoic acid; (A) commercial oral suspension; (B)* **2** *synthesized from reaction of* **1** *with citrate buffer and sodium saccharin; (C)* **2** *synthesized from reaction of* **1** *with citrate buffer only. The two peaks just preceding* **2** *are the solvent, dimethylformamide, and a solvent impurity.*

Table I—Percent of Labeled Amount of Nitrofurantoin in Replicate Assays of Commercial Oral Suspensions[a]

Lot No.	Method			
	USP		HPLC	
1889	96.0	96.5	95.3	95.4
2206	94.2	95.0	94.0	93.8
2727	95.7	95.5	95.4	96.0
2864	94.6	96.7	96.8	95.9
3334	96.4[b]		97.4[c]	

[a] Manufactured by Eaton Laboratories, Inc., Puerto Rico. [b] Average of 14 assays; CV = 1.5%. [c] Average of 10 assays; CV = 0.7%.

the synthesis of the sodium salt of **2** (Fig. 1B), attributed to the citrate buffer (Fig. 1C). As the reaction time increased, the concentration of **2** increased at the expense of **1**, as observed by TLC and HPLC.

The decomposition of **1** with citrate buffer was used to develop a convenient synthesis of **2**. Although the yield of the proposed synthesis is low (~14%), the synthesis is a one-step procedure that starts from inexpensive and readily available nitrofurantoin (**1**) and thus compares favorably with those described in the literature. This procedure avoids the preparation of the starting materials, the oxime of 5-nitro-2-furfural[11] and hydrazoacetic acid, used in the literature method.[4] Since the isolation of hydrazoacetic acid[10,17] was slow and was accompanied by a serious loss of product, the literature method[4] was modified to avoid the isolation steps.

Reversed-phase columns from different manufacturers were used with Procedures I and II, and both showed satisfactory linearity when tested with, respectively, **1** at 50, 100, 150, 175, and 200% of the labeled amount of **1**, and **2** at 0.5, 1.0, and 2.0% of the concentration of **1**. Five recoveries of **1** (5 mg/mL) from a placebo formulation by Procedure I ranged from 99.1 to 100.8%. With **1** (5 mg/mL) and **2** (50 μg/mL) added to the placebo, five recoveries of **2** by Procedure II ranged from 99.1 to 101.3%. A commercial sample that contained **2** (20 μg/mL) was spiked with an additional 50 μg/mL; Procedure II recovered 98.9% of **2**. When five lots of commercial nitrofurantoin oral suspension were assayed for **1** by the compendial method[5] and Procedure I, the results showed good agreement (Table I).

Because the impurity **2** had not yet been identified and synthesized, its amount was estimated from the chromatogram by comparing the areas of **2** and **1**. The concentrations of **2** were higher in older lots of suspension and ranged from 300 to 160 μg/mL (lot expiration dates January 1981 and May 1982, respectively). A current lot (expiration date January 1985) was analyzed by the subsequently developed Procedure II and contained 20 μg of **2**/mL.

References and Notes

1. Harvey, S. C.; Ravin, L. J. in "Remington's Pharmaceutical Sciences"; Osol, A., Ed.; Mack Publishing Co.: Easton, PA, 1980; pp 1160, 1365–1367.
2. Narbutt-Mering, A. B. *Acta Pol. Pharm.* **1980**, *37*, 301; *Chem. Abstr.* **1981**, *94*, 109228.
3. Cadwallader, D. E.; Jun, H. W. in "Analytical Profiles of Drug Substances," vol. 5; Florey, K., Ed.; Academic Press: New York, NY, 1976; pp 358 and 360.
4. Swirska, A. *Acta Pol. Pharm.* **1959**, *16*, 1; *Chem. Abstr.* **1959**, *53*, 11760.
5. "U.S. Pharmacopeia", 20th rev.; U.S. Pharmacopeial Convention: Rockville, MD, 1980; p 550.
6. Aufrere, M. B.; Hoener, B.; Vore, M. E. *Clin. Chem.* **1977**, *33*, 2207.
7. Hoener, B.; Wolff, J. L. *J. Chromatogr.* **1980**, *182*, 246.
8. "Duolite Ion-Exchange Manual", Chemical Process Company: Redwood City, CA, 1960; pp 23–31.
9. "Duolite Technical Sheet 105," Diamond Shamrock: Redwood City, CA, 1976; p 1.
10. Carmi, A.; Pollak, G.; Yellin, H. *J. Org. Chem.* **1960**, *25*, 44.
11. Wright, G. F.; Gilman, H. *J. Am. Chem. Soc.* **1930**, *52*, 2550.
12. Ware, E. *Chem. Rev.* **1950**, *46*, 403.
13. Mouneyrat, A. *Ber. Dtsch. Chem. Ges. II* **1900**, *33*, 2393.
14. Boyd, W. J.; Robson, W. *Biochem. J.* **1935**, *29*, 542.
15. Wheeler, H. L.; Hoffman, C. *Am. Chem. J.* **1911**, *45*, 568.
16. Block, R. J. *Chem. Rev.* **1946**, *38*, 501.
17. Darapsky, A.; Prabhaker, M. *Ber. Dtsch. Chem. Ges. II* **1912**, *45*, 1662.

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