plus its associated impurity). This was done for the purpose of evaluating the relationship between the concentration of TIBA-^{14}C and the products resulting from the photodisintegration. The unidentified compound(s) remaining at the origin were treated in an analogous manner.

The trace of unidentified impurities (spot 1) remaining at the origin increased in quantity to almost 8% of the total radioactivity at the end of 72 hr. of exposure and to 46% after 8 weeks. The quantity of TIBA-^{14}C (spot 2) decreased to 84% at the end of 72 hr. of exposure and to 7% at the end of 8 weeks. Spot 3 of the control (0 time) was identified as 2,5-diiodobenzoic acid. Spot 3 increased to 3% at the end of 72 hr. and 11% at the end of 8 weeks. *o*-Iodobenzoic acid, using the petroleum ether–propionic acid system for separation, has an R_f value very close to that of 2,5-diiodobenzoic acid and may be one of the impurities associated with it. Spot 4 in the control (0 time) was identified as 3,5-diiodobenzoic acid. This compound along with other products of photodisintegration associated with it increased to 5% at the end of 72 hr. and 36% at the end of 8 weeks. *m*-Iodobenzoic acid and also benzoic acid have R_f values using the above solvent system close to that of 3,5-diiodobenzoic acid and are probably impurities associated with the latter. At subsequent time intervals additional unidentified products from the degradation of TIBA-^{14}C became apparent.

CONCLUSIONS

2,3,5-Triiodobenzoic acid in a 2.1×10^{-2}% aqueous suspension is photochemically degraded by ultraviolet light. Ninety-three, 88, 84, 69, and 7% of TIBA are recovered from solution after 24, 48, 72 hr., 1 and 8 weeks of exposure, respectively. Two products of degradation were identified as 2,5- and 3,5-diiodobenzoic acid. From this study, it can be concluded that TIBA might be degraded in a like manner by sunlight after its application to plants.

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Keyphrases

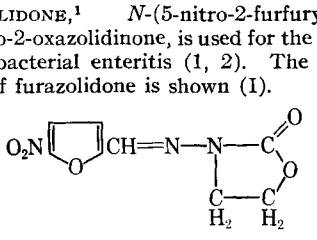
^{14}C-Carboxyl 2,3,5-triiodobenzoic acid
UV photodegradation of ^{14}C-carboxyl 2,3,5-triiodobenzoic acid
Degradation products of ^{14}C-carboxyl 2,3,5-triiodobenzoic acid
TLC separation
Liquid scintillation spectrometry—analysis

Method Specific for Determination of Furazolidone in Urine: Evidence for Drug-Related Metabolites

By R. D. HOLLIFIELD and JOHN D. CONKLIN

Furazolidone, *N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone, is a chemotherapeutic drug used orally for the treatment of bacterial enteritis. A new, more specific analytical method for the determination of furazolidone in urine is described. Utilizing this procedure, furazolidone was not detected in urine samples collected from dogs and humans following oral administration of the drug. Evidence is provided for the presence of drug-related metabolites.

FURAZOLIDONE,[1] *N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone, is used for the oral treatment of bacterial enteritis (1, 2). The structural formula of furazolidone is shown (I).



I

Previously, urinary concentrations of furazolidone were measured either by bioassay (1) or by the method of Nakamura and Inoue (3), which is based on the conversion of the drug to 5-nitrofurfural phenylhydrazone. A new analytical procedure, more specific for the determination of furazolidone in urine, is described in this report. Results obtained by this procedure and by the Nakamura and Inoue method are presented regarding furazolidone concentrations in dog and human urine following oral drug administration.

EXPERIMENTAL

Drug Administration—Micronized furazolidone (about 5 μ or less) in gelatin capsules was administered orally to unfasted, adult, male beagle dogs at 1.25 mg./Kg. q.i.d. at 4-hr. intervals (5 mg./Kg./day). Urine samples were then collected by catheterization at selected intervals. Furazolidone as a

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[1] Eaton Laboratories' trademark for furazolidone is Furoxone.

TABLE I—RECOVERIES OF FURAZOLIDONE FROM DOG AND HUMAN URINE

Fluid	Range of Control	Absorbance at 600 mμ[a] — Furazolidone, mcg./ml. 2	5	10	30	50	Av. Recovery[b] Mean ± S.D.
Water	0.000–0.002	0.030	0.069	0.138	0.418	0.719	
Dog urine	0.007–0.011	0.029	0.066	0.138	0.427	0.708	98.6 ± 2.6
Human urine	0.005–0.008	0.032	0.071	0.140	0.420	0.718	102.2 ± 2.3

[a] Control corrected data based on a mean from at least three samples. [b] Based on furazolidone concentrations in water.

TABLE II—DETERMINATION OF FURAZOLIDONE CONCENTRATIONS IN DOG URINE

Dog	Dose[a] mg./Kg./day	Interval, hr.	Method — DMF–DDAH, mg./L.	Nakamura and Inoue, mg./L.
A	5	0–4	None detected	2.7
		4–8	None detected	15.6
		8–12	None detected	9.0
		12–24	None detected	0.1 (1.2)[b]
B	5	0–4	None detected	3.4
		4–8	None detected	8.9
		8–12	None detected	6.5
		12–24	None detected	1.6 (2.4)[b]

[a] Micronized furazolidone (about 5 μ or less) in gelatin capsules was administered orally at 1.25 mg./Kg. q.i.d. every 4 hr. [b] Represents the percentage of dosed material convertible to 5-nitrofurfural phenylhydrazone recovered in the urine within 24 hr.

100-mg. tablet[2] or as 100 mg. of macrocrystalline drug (about 75 μ) in a gelatin capsule was administered orally to healthy adult, human males q.i.d. at 4-hr. intervals (5 mg./Kg./day for an 80-Kg. adult). Voided urine specimens were then obtained at selected intervals.

Drug Analysis—The reagents include: crystalline furazolidone (Eaton Laboratories); saturated ammonium sulfate solution, pH 6.0, special enzyme grade (Mann Research Lab., Inc.); spectro-quality nitromethane and *N*,*N*-dimethylformamide (DMF), reagent grade (Matheson, Coleman, and Bell); and p-diisobutylcresoxyethyoxyethyl dimethylbenzyl ammonium hydroxide[3] (DDAH) 1 *M* in methanol (443 Gm./L. methanol, Packard Instrument Co., catalog No. 6003005). One milliliter of this DDAH solution is diluted to 25 ml. with DMF to produce a 0.04 *M* solution. A Beckman DU spectrophotometer was used to measure absorbance.

Reference drug solutions are prepared by dissolving 50 mg. of furazolidone in 50 ml. of DMF and diluting this with water to obtain the necessary drug concentrations. One milliliter of urine and 2 ml. of water are mixed together, 3 ml. of saturated ammonium sulfate solution added, and the contents mixed. Ten milliliters of nitromethane is then added, the contents mixed vigorously for 1 min. and centrifuged for 10 min. at 2,000 r.p.m. Eight milliliters of the nitromethane extract (top layer) is transferred to a test tube containing 1 Gm. of sodium sulfate, and the contents are mixed vigorously for 1 min. to dry the solvent. Six milliliters of the nitromethane extract is removed and placed in a 50-ml. flask, and the solvent is evaporated under vacuum at 50° using a flash evaporator. Following evaporation, the residue is dissolved in 3 ml. of DMF. Exactly 2.9 ml. of this solution is transferred to a test tube, 0.5 ml. of 0.04 *M* DDAH in DMF (freshly prepared) added, and the absorbance determined at 600 mμ. Since the presence of water inhibits the color formation, water contamination of the DMF should be avoided. It is recommended that the absorbance of each sample be measured within 10 min. following addition of the DDAH solution since the color formed deteriorates slowly with time.

Some of the urine samples were examined chromatographically. The sample was extracted with nitromethane, the extract spotted on Whatman No. 1 paper, and subjected to ascending paper chromatography for 15 hr. at room temperature. After drying, the papers were examined under UV light and the R_f of each spot determined. The solvent systems used were: 95% ethanol, *n*-butanol, and either 0.5 *N* acetic acid or ammonium hydroxide (1:4:1).

RESULTS AND DISCUSSION

DMF-DDAH Procedure—The furazolidone–DDAH complex in DMF exhibits an absorbance maximum at 600 mμ. The standard curve for this color complex in DMF follows Beer's law to 50 mg./L. The drug recoveries from dog and human urine (Table I) indicate that the reference standards and internally corrected standards are identical, and that the sensitivity of the method is at least 2 mg./L.

Urinary Drug Concentrations—The method described was applied to the determination of furazolidone in urine samples collected after drug administration. Micronized furazolidone (about 5 μ or less) in gelatin capsules was administered orally to dogs. Furazolidone as a 100-mg. tablet or as macrocrystalline drug (about 75 μ) in gelatin capsules was administered orally to human subjects. Urine samples were collected at designated intervals. As shown by the results (Tables II and III), furazolidone was not detected in any of the urine samples analyzed by the DMF–DDAH method.

For comparison, the urine samples were also analyzed for drug by the Nakamura and Inoue procedure (3), which revealed the presence of material(s) convertible to 5-nitrofurfural phenylhydrazone. It has been reported that 5-nitrofurfural phenylhydrazone in toluene exhibits a red band when eluted on a column of aluminum oxide (4). Some of the urine samples (Tables II and III) were subjected to the colorimetric procedure of Buzard *et al.* (5), in which furazolidone is converted to 5-nitrofurfural phenylhydrazone and extracted with toluene. The characteristic red band was observed when these toluene extracts were examined on aluminum oxide columns, providing additional evidence for the presence of material(s) convertible to 5-nitrofurfural phenylhydrazone in dog and human urine following furazolidone dosage.

Recently, interest has been directed toward drug particle size and its related effects *in vivo* with regard to the absorption of nitrofuran derivatives (6). Relative to this, it was observed that greater amounts of material(s) convertible to 5-nitrofurfural phenylhy-

[2] Furoxone tablet.
[3] Hyamine hydroxide.

TABLE III—DETERMINATION OF FURAZOLIDONE CONCENTRATIONS IN HUMAN URINE

Subject	Dose[a] 400 mg./Day	Interval, hr.	Method: DMF–DDAH mg./L.	Method: Nakamura and Inoue, mg./L.
A	Tablet	0–4	None detected	7.7
		4–8	None detected	54.6
		8–12	None detected	25.8
		12–24	None detected	12.0 (6.6)[b]
B	Tablet	0–4	None detected	20.4
		4–8	None detected	26.1
		8–12	None detected	44.1
		12–24	None detected	9.9 (8.7)[b]
C	Gelatin capsule	0–4	None detected	8.1
		4–8	None detected	15.6
		8–12	None detected	3.1
		12–24	None detected	9.5 (3.0)[b]
D	Gelatin capsule	0–4	None detected	7.0
		4–8	None detected	5.2
		8–12	None detected	13.6
		12–24	None detected	8.5 (3.1)[b]

[a] Furazolidone as a 100-mg. tablet or as 100 mg. of macrocrystalline drug (about 75 μ) in a gelatin capsule was administered orally q.i.d. every 4 hr. [b] Represents the percentage of dosed material convertible to 5-nitrofurfural phenylhydrazone recovered in the urine within 24 hr.

TABLE IV—PAPER CHROMATOGRAPHY OF DOG AND HUMAN URINE

Sample	R_f Values: Acidic, Dog	Acidic, Human	Basic, Dog	Basic, Human
Control urine	0.14	0.12	0.12	0.16
	0.37	0.36	0.31	0.35
	0.58		0.59	
		0.74		
Control urine plus furazolidone	0.16	0.12	0.17	0.16
	0.36	0.37	0.30	0.34
	0.59		0.58	
	0.66[a]	0.68[a]	0.65[a]	0.67[a]
		0.73		
Exptl.[b]	(0.05)[c]	(0.05)	(0.07)	(0.07)
	0.15	0.13	0.19	0.13
	0.37	0.38	0.29	0.35
	(0.51)	(0.55)	(0.43)	(0.44)
	0.59		0.58	
		0.74		

[a] R_f value for furazolidone. [b] Dog *A* (4–8 hr.) Table II, subject C (4–8 hr.) Table III. [c] Figures in parentheses represent drug-related metabolites.

drazone were recovered in the urine of humans administered the furazolidone tablet than were found in the urine of subjects administered the macrocrystalline drug (Table III).

Chromatography—A chromatographic comparison of urine collected from dogs and humans administered furazolidone and of urine to which furazolidone had been added was conducted. Although furazolidone was detected when added to the urine (Table IV), the drug was not detected in either dog or human urine following oral furazolidone administration. However, the presence of two drug-related metabolites, one yellow and the other orange in appearance under UV light, in both dog and human urine, was established.

Following chromatography the drug-related spots were eluted and their absorbance spectra determined. These eluates were also analyzed chemically. When the spot representing drug added to urine (R_f 0.65–0.68, Table IV) was eluted, it exhibited an absorbance spectrum with a maximum at 367 mμ in water, which is characteristic of that reported for furazolidone (1). This eluate yielded the expected blue color when subjected to either the DMF–DDAH or Nakamura and Inoue procedures. As shown in Table V, each of the metabolites exhibited an absorbance spectrum with a maximum near 415 mμ in water. Apparently, the spot which appears orange under UV light (Table V) represents one of the material(s) in dog and human urine which is convertible to 5-nitrofurfural phenylhydrazone. The presence of drug-related metabolites which absorb near 415 mμ in the urine of animals administered certain nitrofuran derivatives has been reported previously (7–9).

On the basis of spectral analysis, the yellow material appears to be present in a greater amount than the orange material in dog urine, while the orange material is present in greater amounts than the yellow material in human urine. This indicates that although the two drug-related metabolites are each present in dog and human urine, they apparently are present in different relative amounts. This may explain the twofold difference observed between dog and man (Tables II and III) in the percent excretion of dosed micronized material convertible to 5-nitrofurfural phenylhydrazone.

Under *in vitro* conditions, furazolidone exhibits significant inhibitory activity against *Escherichia coli* (1). Urine samples collected from dogs and humans following furazolidone administration demon-

TABLE V—CHARACTERISTICS OF THE DRUG-RELATED URINARY METABOLITES

Sample	R_f Value[a]: Acidic	Basic	Appearance: White Light	UV Light	Absorbance Max. in Water, mμ	Chemical Assay: DMF–DDAH Method	Nakamura and Inoue Method
Dog urine	0.05	0.07	Yellow	Yellow	415	No color	No color
	0.51	0.43	Yellow	Orange	415	No color	Blue color
Human urine	0.05	0.07	Yellow	Yellow	415	No color	No color
	0.55	0.44	Yellow	Orange	415	No color	Blue color

[a] See Table IV.

strate little activity against *E. coli* (1), suggesting that only small amounts of furazolidone are excreted in dog and human urine. This also indicates that the urinary drug-related metabolites observed in the present study are not significantly active against *E. coli*.

In summation, when urines collected from dogs and humans administered furazolidone orally were analyzed by a new analytical procedure, furazolidone was not detected. Chromatographic examination of these urine samples verified this conclusion and also revealed the presence of two drug-related metabolites in both dog and human urine.

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Keyphrases

Furazolidone
Urine–furazolidone analysis procedure
Metabolites, furazolidone–analysis in urine
Colorimetry–analysis
Paper chromatography–analysis

Fractionation of Fatty Acids of *Cucurbita maxima* Seed Oil With Urea

By J. P. TEWARI and M. C. SRIVASTAVA

The mixed fatty acids of *Cucurbita maxima* seed oil have been fractionated by liquid–solid countercurrent distribution with urea. The percentage fatty acid composition of oil is: palmitic, 21.5; stearic, 8.4; oleic, 27.0; and linoleic, 43.10.

UNDER the Indian Council of Medical Research inquiry on anthelmintic activity of *Cucurbita maxima* seeds (family, *Cucurbitaceae*), the anthelmintic activity and the chemotherapeutic actions of aqueous, alcoholic, and ethereal extracts of the decorticated seeds of *C. maxima* have been reported (1, 2).

A large amount of the oil was obtained as a by-product during the defatting of the seeds prior to the isolation of cucurbitin. In view of the importance of this oil in the Indian system of medicine (3, 4) the mixed fatty acids of *C. maxima* oil were fractionated by the liquid–solid countercurrent distribution of fatty acids with urea employing the method of Sumerwell (5).

The results of fractionation have been recorded in Table I and agree closely with those obtained by Chowdhury *et al.* spectrophotometrically (6).

TABLE I—LIQUID–SOLID COUNTERCURRENT DISTRIBUTION OF *C. maxima* FATTY ACIDS WITH UREA

S. No. of Fraction	Wt. of Fraction	S.E.	I.V.	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid
1	3.28	205.2	0.82	1.20	2.04	0.03	...
2	2.61	215.8	2.42	2.26	0.28	0.07	...
3	2.08	215.2	2.52	1.72	0.19	0.18	...
4	1.56	216.2	6.92	1.38	0.06	0.12	...
5	...	...	...	...	...	...	...
6	0.61	195.4	90.1	...	...	0.61	...
7	...	...	...	...	...	...	...
8, 9, 10	0.91	196.4	90.2	...	...	0.91	...
11	4.85	197.8	121.2	...	...	3.20	1.65
Raffinate	14.60	198.5	162.0	...	...	3.10	11.50
Total	30.50	...	...	6.56	2.57	8.22	13.15
Percentage of acids	...	...	...	21.5	8.4	27.0	43.1
Percentage of acids by Chowdhury (6)	...	...	...	←——29.9——→		26.4	43.7

EXPERIMENTAL

The oil from the seeds of *C. maxima* was saponified and fatty acids were obtained from the soap after removing the unsaponifiable matter. The mixed fatty acids (30.5 Gm. I.V., 103.2; N.V., 198.2) were fractionated by liquid–solid countercurrent distribution of fatty acid with urea employing the method

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