probably traceable to sampling procedures and not to errors within the method itself. The average for the 30-day period, 6.38 γ per gram, was in good agreement with the value claimed by the manufacturer, 5.73 γ per gram, when it is taken into account that 30 samples were taken, each from a separate day's run on a broiler feed formula in a commercial feed operation.

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FEED ADDITIVES ANALYSIS

Microdetermination of the Medicaments Furazolidone and Nitrofurazone

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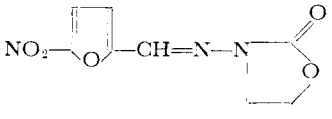
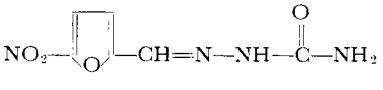
Colorimetric procedures allow the assay of a feed containing a mixture of furazolidone and nitrofurazone. *N,N*-Dimethylformamide is used as the solvent for the compounds and as the medium in which the color of furazolidone is developed. The color is developed with nitrofurazone in mixture of alcohol and dimethylformamide. Furazolidone develops a blue and nitrofurazone a pink-orange color in the presence of alkali. A chromatographic separation of these feed medicaments is described.

FURAZOLIDONE, *N*-[(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone], is represented by the following formula:



Nitrofurazone, 5-nitro-2-furaldehyde semicarbazone, is represented by:



Several methods of determining furazolidone and nitrofurazone have been published (*1–3*). Some require reduction of the active materials for a blank reading in the absence of control samples. These procedures give variable results, partly inherent and partly due to the limited solubility of the compounds in the solvents used. Other methods give good results, but are somewhat more complicated and time-consuming than the procedure proposed here. No method for separating the components of a mixture of these medicaments has been published. Porter (*4*) used dimethylformamide as a solvent in a color test for mono- and dinitro compounds. He investigated aromatic compounds and utilized tetraethylammonium hydroxide, but did not include nitrofurans or other heterocyclics.

As these compounds are finding wide acceptance in the feed industry, a rapid procedure for routine laboratory analysis is of prime importance. This procedure requires but a few steps and reagents.

Materials and Equipment

Extraction tubes, 12 inches, made from 20-mm. glass tubing, drawn to a tip.

Chromatographic columns, a 50-ml. buret or one about 12 × 450 mm.

Spectrophotometer, suitable for reading at 490 and 600 mμ.

Skellysolve B is a satisfactory petroleum hydrocarbon solvent. *N,N*-Dimethylformamide, 95% ethyl alcohol, and potassium hydroxide (1*N*) in 50% alcohol with water.

Aluminum oxide, suitable for chromatographic adsorption. Each lot should be checked for recovery of the medicaments. An acid wash of the alumina generally corrects any difficulties.

Crystalline furazolidone and nitrofurazone, obtained from Eaton Laboratories, Division of Norwich Pharmacal Co., Norwich, N. Y., and Bifuran from Hess and Clark, Inc., Ashland, Ohio.

Procedures

Standards. To prepare stock solutions of each compound, weigh exactly 0.1000 gram and transfer to a 100-ml. volumetric flask, using dimethylformamide. One milliliter of this (1.0 mg.) is equivalent to the amount of medicament in 9.1 grams of feed at 0.011% concentration. Prepare other dilutions to correspond to various amounts of medicament in the feed or concentrate. Standards are more comparable to samples if the standard curves are made by using known amounts of medicament added to a blank feed.

Pre-extraction. All feeds should be mechanically ground to pass a 20-mesh sieve. As furazolidone is not very soluble in Skellysolve B, it is convenient to use it to pre-extract the feeds to remove interfering material. Add 9.1 grams of feed to an extraction tube containing, first, a glass wool plug, and then 1 cm. of Hyflo Super-Cel in the tip. Allow 50 ml. of Skellysolve to pass through the column. If necessary, ap-

Table I. Recovery from Poultry Feed

Concn. in Feed, %	Sample No.	Concn. Found, %	% Recovery
		Furazolidone	
0.0055	1	0.0053	96.4
	2	0.0056	101.8
	3	0.0054	98.2
	4	0.0055	100.0
		Av. 0.00545	99.11
0.011	11	0.0106	96.4
	12	0.0107	97.3
	13	0.0108	98.2
	14	0.0105	95.5
		Av. 0.01065	96.8
0.022	21	0.0210	95.5
	22	0.0212	96.2
	23	0.0210	95.5
	24	0.0214	97.3
		Av. 0.0212	96.2
		Nitrofurazone	
0.0055	31	0.00550	100
	32	0.00550	100
	33	0.00495	90
	34	0.00495	90
		Av. 0.00523	95
0.011	41	0.0110	100
	42	0.0110	100
	43	0.0110	100
	44	0.0117	106
	45	0.0117	106
	46	0.0099	90
		Av. 0.011	100
0.022	51	0.0238	108
	52	0.0231	105
	53	0.0233	106
	54	0.0238	108
	55	0.0198	90
		Av. 0.0227	103

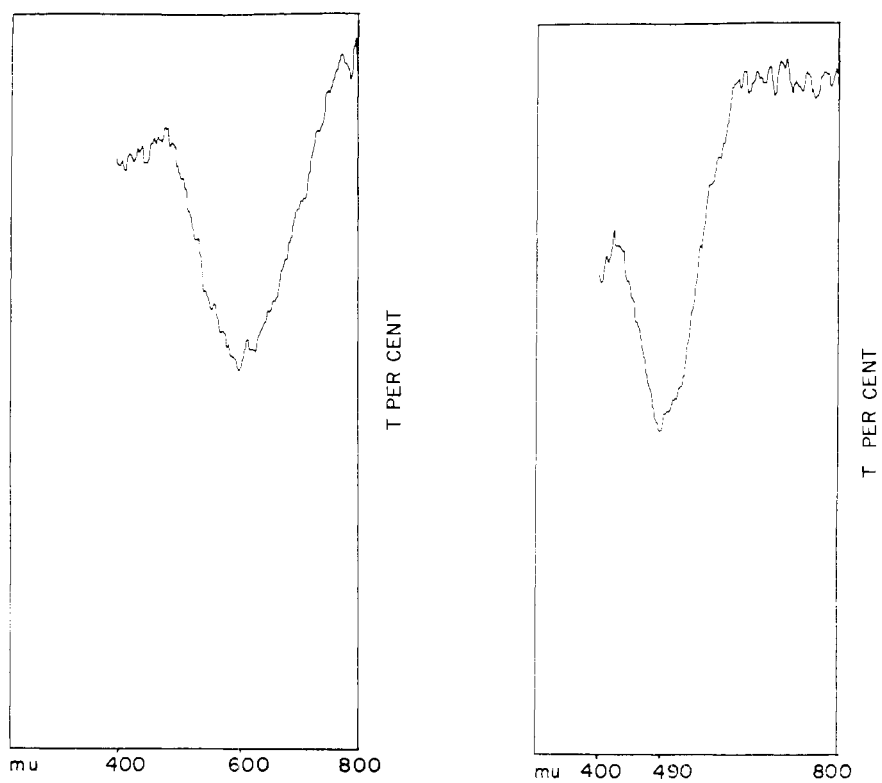


Figure 1. Absorption curves

Left. Furazolidone
Right. Nitrofurazone
Lead sulfide cell
Sensitivity, 300
Range, 0 to 100%

Time constant, 0.1
Scanning time, 10
Scale expansion × 2
Wave length scon, 830 to 400 mμ

ply gentle suction to the column. After all the solvent has passed through, apply suction until the feed is dry of solvent (when the column returns to room temperature). Feeds containing nitrofurazone need not be extracted with Skellysolve. Weigh the feed directly into an Erlenmeyer flask and treat as follows:

Extraction. Put the dry feed into a 250-ml. Erlenmeyer flask with 100 ml. of dimethylformamide. Stopper the flask and agitate frequently for 30 minutes. Filter the sample and take a 10-ml. aliquot for analysis.

As the compounds are sensitive to sunlight or fluorescent light, the operation should be carried out only under incandescent light.

Chromatography. Prepare an adsorption column, adding first a glass wool plug and then 10 grams of aluminum oxide. Tap to settle the oxide and put a small glass wool plug on top to act as a splash barrier. Pressure or vacuum may be used to speed the flow of liquid. Introduce 10 ml. of the filtrate onto the dry column, and just as the last of it goes into the oxide, add 30 ml. of dimethylformamide. If furazolidone is present, the 30 ml. of dimethylformamide will wash it off the column. Collect the eluate for analysis and color development.

If nitrofurazone is present, a red-orange band will form just below the surface of the alumina. Do not allow nitrofurazone to remain on the column any longer than necessary before eluting. After the 30 ml. of dimethylformamide has passed through, discard the eluate (or save and test for furazolidone, if a mixture of the two is suspected). The nitrofurazone can be removed from the column by ethyl alcohol (30 ml.). Collect the alcohol fraction in a 50-ml. volumetric flask. This fraction contains a small amount of dimethylformamide and the alcohol that passes through the column. The components of Bifuran may be determined separately and quantitatively.

Color Development. Make the resultant solutions, whether nitrofurazone or furazolidone, to the mark, using dimethylformamide. After mixing remove a portion for use as the blank reading for the sample. Add 1 ml. of 1*N* potassium hydroxide in 1 to 1 ethyl alcohol–water to the balance and shake. The color developed is a blue for the furazolidone and a pink-orange for the nitrofurazone. The furazolidone absorbs at 600 mμ and the nitrofurazone at 490 mμ. Read the samples as soon as the color is developed. Never wait more than 2 minutes after color development to read the color, although the colors remain stable up to 15 minutes in the absence of light.

Tables of recoveries of these compounds are shown (Tables I and II)

for amounts equivalent to concentrations expected in prepared feeds and premixes. Figure 1 shows absorption curves for furazolidone and nitrofurazone, made by a Beckman DK-2 ratio-recording spectrophotometer.

Discussion

The specificity of the color reaction with respect to solvent was investigated. No color was developed with the compounds being studied in the presence of potassium hydroxide and any of the following solvents: acetone, ethyl ether, nitromethane, dioxane, chloroform, carbon tetrachloride, and 1,1,1-trichloroethane.

Several other medicaments (vitamin A, Enheptin, Nitrophenide, diethyl stilbestrol, arsanilic acid, sulfaquinoxaline, phenothiazine, Nicarbazin, and Nitrosal) were dissolved in dimethylformamide and tested for any color development. None developed a color that would interfere with the method described. Nitrosal gave a yellow solution in dimethylformamide, but was not changed when the potassium hydroxide solution was added. Enheptin developed a light yellow and Nicarbazin a deep yellow color after alkali was added. Nitrophenide developed a brownish pink color when the alkali was added.

The use of dimethylformamide obviates the possibility of an incomplete extraction, as the compounds sought are soluble in this solvent, compared to alcohol and other mixtures used in other methods.

Several brands of chicken and turkey feeds, both mashes and pelleted, were mixed, ground together, and remixed. This composite sample was used throughout the experimentation. None of the feeds in this mixture contained any of the medicaments (except vitamin A) mentioned earlier in the paper.

Table II. Bifuran Recovery from Poultry Feed

Sample No.	Nitrofurazone, Mg.		Furazolidone, Mg.	
	Added	Recovered	Added	Recovered
61	0.556	0.556	0.0825	0.082
62	0.556	0.560	0.0825	0.082
63	0.556	0.554	0.0825	0.100
		Av. 0.556		0.088
71	1.120	1.12	0.165	0.175
72	1.120	1.13	0.165	0.180
73	1.120	1.17	0.165	0.180
		Av. 1.14		0.178
81	2.240	2.30	0.330	0.320
82	2.240	2.10	0.330	0.330
83	2.240	2.24	0.330	0.350
		Av. 2.21		0.333
91	3.360	3.50	0.495	0.480
92	3.360	3.36	0.495	0.490
93	3.360	3.20	0.495	0.525
		Av. 3.35		0.498

Nitrofurazone develops a color similar to furazolidone in pure dimethylformamide solution, when a few drops of potassium hydroxide solution are added. The color is characteristic for nitrofurazone, but was not used for this method because of poor recoveries after chromatography and increased manipulations to remove the alcohol. Nitrofurazone gives a red solution in acetonitrile on addition of an alkali. Nitrofurazone is only slightly soluble in acetonitrile, however.

Feeds that contain mixes of furazolidone, nitrofurazone, and 3-nitro-4-hydroxyphenylarsonic acid may also be analyzed by this procedure. The chromatography step holds the 3-nitro-4-hydroxyphenylarsonic acid on the column. Solvents such as alcohol, acetone, or diethyl ether do not remove this material.

The phenylhydrazine method of Buzard (*1*) may be applied to the solutions after the components of the mixture have been resolved.

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HEAT EFFECTS ON MILK

Review of Organic Chemical Effects of Heat on Milk

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Many heat-induced changes in milk and its products can be studied appropriately by an organic chemical approach. Research progress using such an approach is discussed in terms of lactose-protein interaction (browning) and flavor changes in the fat and nonfat phases of milk. The need for research concerning effects of heat on milk fat is emphasized. To make milk a more flexible and useful raw material and to overcome some of its tendencies toward chemical deterioration, custom manufacture of milk components, such as the fat and protein, should be more extensively developed by the dairy industry.

PRACTICALLY ALL MILK and all its products are heat-processed to some degree. In fact, many dairy products gain their identity through one or a combination of processing steps in which heat treatment is inherent: pasteurization, homogenization, preheating (forewarming), mixing, blending, condensing, superheating, sterilization, and drying. Such measures are not without effect on milk, a delicately balanced biochemical system. Because milk contains considerable organic matter, some of these effects can be investigated to advantage from the standpoint of organic chemistry.

Appreciation of classical organic chemistry must be modified somewhat in applying it to a food system. It is not clear what may be reacting with what when a complex medium such as milk is heated. Thus understanding of re-