Determination of Nitrofurazone in Milk

GARY E. PAAR
Agricultural Experiment Station, Auburn University, Auburn, Ala.

A short, convenient, photometric procedure for the determination of nitrofurazone (5-nitro-2-furaldehyde semicarbazone) has been developed for use in residue analysis. By precipitation of milk protein with sodium tungstate and sulfuric acid, concentrations of the above nitrofuran derivative can be estimated down to the 0.5-p.p.m. level.

THE DETERMINATION of drug residues in samples of biological origin presents many problems. Materials of this nature often contain a multitude of substances that must be eliminated to provide an accurate estimation of the desired ingredient. In addition, micromethods are appropriate since public and governmental interest is at a current high regarding residues in food products designated for human consumption. The object of this work was to establish a method of analysis which would result in adequate sensitivity and still not require an excess of time because the number of samples to be processed for residue experiments is usually high.

A survey of the available literature led to the development of an adequately sensitive method involving conversion of nitrofurazone to the corresponding phenylhydrazone with simultaneous precipitation of protein and subsequent toluene extraction of the phenylhydrazone. The absorbance was then read with a suitable spectrophotometer. Spectra of the toluene extracts of milk samples containing the drug and treated by this procedure were similar to those obtained from plasma (*3*), yielding an absorption maximum at 430 mμ after the spectrum from an unmedicated sample had been subtracted. This method after adaptation in this laboratory gave satisfactory results on pasteurized, homogenized milk, but poor precision on raw milk samples from a number of sources.

An analytical method for nitrofuran derivatives excreted in urine involves the determination of the absorbance of a sample at the maximum in the ultraviolet region (*5*). The absorption spectrum of nitrofurazone in distilled water is given in Figure 1. The recommended procedure involves precipitation of milk protein and determination of the absorbance at the 375-mμ maximum.

Experimental

Reagents. Sodium tungstate dihydrate, 10% (Merck, Folins' grade).

Sulfuric acid, 0.3*N*, prepared by volume from concentrated acid (C.P. grade).

Crystalline Furacin (nitrofurazone, Eaton Laboratories, The Norwich Pharmacal Co., Norwich, N. Y.).

Spectrophotometer, Beckman, Model DU or equivalent.

Centrifuge, International, size 1, Model SBV.

Recommended Procedure. To a 50-ml. Erlenmeyer flask containing 18 ml. of 0.3*N* sulfuric acid, add 25 ml. of milk to be tested, from a pipet, with thorough agitation. Let the suspension stand for 5 minutes and add 5 ml. of sodium tungstate solution, 0.5 ml. at a time with swirling, until all is added. Mix well by shaking; pour the contents into a 50-ml. polyethylene tube and centrifuge for 10 minutes at 1500 r.p.m. Filter the centrifugate through Whatman No. 3 paper, rinse the cuvette with a small portion of the filtrate, refill, and read the absorbance from 360 to 390 mμ in 5-mμ intervals with a suitable spectrophotometer against distilled water.

Spectrophotometer cuvettes used in this procedure must be kept scrupulously clean, since the filtrates involved deposited a cloudy film on the inside walls of the cuvette optical surface when left in a partially or completely empty condition between successive measurements.

Table I. Recovery Data

Sample from Cow, No.	Apparent Levels in Solutions Made up to 5.01 P.P.M.	Recovery, %	Apparent Levels in Solutions Made up to 10.02 P.P.M.	Recovery, %
		Procedure I		
74	4.9	98	11.4	114
103	5.4	108	10.0	100
119	...	...	10.7	107
149	5.1	102	10.6	106
174	4.7	94	...	...
181	5.1	102	...	...
183	5.7	114	10.8	108
186	...	...	10.4	104
194	5.0	100	10.8	108
195	...	...	11.5	115
196	4.6	92	11.8	118
198	...	...	11.2	112
204	5.0	100	...	...
209	4.8	96	10.4	104
211	4.4	88	...	...
212	5.6	112	10.4	104
(Standard deviation = 7.8%)			(Standard deviation = 5.4%)	
		Procedure II		
74	5.1	102	9.8	98
85	4.8	96	9.6	96
103	4.7	94	9.6	96
149	4.9	98	9.7	97
174	4.8	96	9.5	95
181	5.1	102	9.8	98
183	5.1	102	10.0	100
194	4.6	92	9.7	97
204	5.0	100	10.0	100
209	5.1	102	9.7	97
211	4.9	98	9.4	94
212	5.1	102	9.6	96
(Standard deviation = 3.6%)			(Standard deviation = 1.8%)	

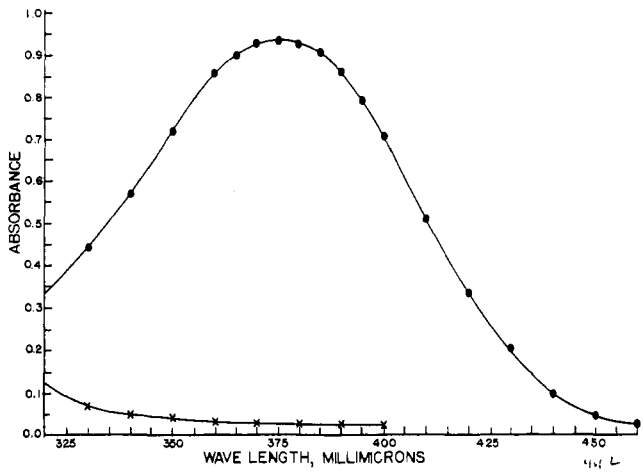

Figure 1. Spectra of nitrofurazone in distilled water (—●—●—) and a typical milk filtrate not containing nitrofurazone after precipitation with sodium tungstate (—X—X—)

Standard solutions of nitrofurazone in milk can be prepared by dissolving 50 mg. of crystalline Furacin in 5 ml. of dimethylformamide and diluting to 500 ml. with distilled water. Solutions in milk are then prepared from this standard at various concentrations.

Results and Discussion

Two methods of determination were evaluated in this experiment. Results obtained by the two procedures are compared in Table I. Procedure I involves addition of phenylhydrazine hydrochloride and concentrated hydrochloric acid to the milk, followed by digestion at 70° C. and subsequent toluene extraction. Procedure II is the sodium tungstate precipitation method used and suggested by the author. Blanks were determined for all reagents, except nitrofurazone.

Appropriate amounts of sodium tungstate and sulfuric acid must be used to ensure a filtrate near neutrality so as to guard against the acid-catalyzed decomposition of nitrofurazone (*2*), even though the precipitating agent works most efficiently in an acidic medium (*1*). Reagents therefore were empirically adjusted with Hydrion test papers to yield a clear filtrate at a pH of approximately 5. Determinations were performed out of contact with fluorescent lighting to avoid possible ultraviolet - induced decomposition. Using this method, the filtrates were sufficiently stable during analysis. Variation in filtrate absorbances over a 4-hour period was within instrument readability.

The variation in results indicated in Table I is presumably due to the variable pigment concentration present in the milk, which contributes to the absorbance at the wave lengths chosen for analysis. Chromatography of toluene extracts obtained using procedure I, on alumina with ethyl acetate–toluene or dimethylformamide-toluene mixtures, removed some pigments; however, enough pigment remained to affect the reproducibility of the results.

Results on an absolute basis were determined by preparing a standard solution of nitrofurazone and using this standard to prepare various nitrofurazone solutions in milk over a 1- to 30-p.p.m. range. The milk solutions were then subjected to the suggested procedure, and a plot of nitrofurazone concentration *vs.* absorbance at 375 mμ was constructed. The data yielded a linear relationship following Beer's law over the range tested. The slope of a typical curve was 0.034 absorbance unit per p.p.m. of nitrofurazone. If the differences in absorbance of the nitrofurazone solutions and the absorbance of blank solutions are plotted against concentration, the resulting curve passes through the origin. Concentrations of nitrofurazone in other milk samples can then be read directly from the standard curve. Unless a definite maximum at 375 mμ is present, the qualitative presence of the drug in the sample cannot be established since all control samples tested in this laboratory exhibited significant absorption ranging from 0.013 to 0.029 absorbance unit at this wave length; however, maxima of medicated samples down to levels of 0.5 p.p.m. were quite apparent with associated absorbance values of approximately 0.020 unit above that of the controls.

Variations in composition of samples of biological origin are the causitive reasons for nonuniformity of results in many types of analyses by chemical or physical measurements. For example, the blank absorbance values of samples and fat content of the milk obtained for sampling showed consistently higher values for the Jersey breed known for its higher fat assay, but attempts at correlating blank absorbance with butterfat percentage or milk density yielded no close quantitative relationship.

To ascertain the effect of these variations, milk samples from Jersey and Holstein cows in various stages of lactation were obtained from the Auburn University Dairy Farm and used to assemble the data in Table I. Somewhat larger errors might be introduced if a considerably atypical milk sample was used in making up the tandard curve. Bottled samples of pasteurized, homogenized milk from the University Dairy were found to be excellent as typical samples for preparing adequately reproducible curves.

The chief advantages of the recommended procedure appear to be a distinct saving in time and a reduction in manipulative error resulting from fewer necessary procedural steps per sample handled, as well as an increase in precision caused by the apparent reduction of background color effect inherent in samples.

Results of experiments determining time of residence for nitrofurazone following intramammary infusion using the suggested procedure and statistical analysis of the data are presented elsewhere (*4*).

The suggested procedure might be applicable to the analysis of milk for other nitrofuran derivatives after establishing their particular absorption maximum; however, before applying the method to samples of different origin, establishment of color contributions by the sample itself would be in order.

Acknowledgment

The author expresses his appreciation for the valuable suggestions of George Hawkins and the assistance of Alice Jana Gore during this study.

Literature Cited

(1) Berkman, S., Henry, R. J., Golub, O. J., Segalove, M., *J. Biol. Chem.* **206,** 937 (1954).
(2) Buzard, J. A., Eaton Laboratories, The Norwich Pharmacal Co., Norwich, N. Y., private communication, September 1960.
(3) Buzard, J. A., Vrablic, D. M., Paul, M. F., *Antibiotics & Chemotherapy* **6,** 702 (1956).
(4) Hawkins, G. E., Paar, G. E., Cannon, R. Y., *J. Dairy Sci.*, to be published.
(5) Paul, H. E., Austin, F. L., Paul, M. F., Ells, V. R., *J. Biol. Chem.* **180,** 345 (1949).

Received for review April 27, 1961. Accepted September 13, 1961. Work supported, in part, by a research grant of the U. S. Public Health Service for the study of secretion rates of various chemotherapeutic agents in dairy cows.