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NOTES

Turbidimetric Method for Assay of Nitrofuran Compounds

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Abstract □ A turbidimetric method for the assay of nitrofuran compounds in urine and serum is described. The results of the assay were subjected to statistical analysis. The extent of inhibition and the form of regression of responses were affected by the concentration of indicator organism, time of incubation, and quantity of serum or urine. Optimal conditions for the assay of nitrofurantoin and furazolidone were determined.

Keyphrases □ Nitrofuran compounds — turbidimetric assay in urine and serum, using *S. faecalis* as test organism □ Turbidimetric assay—nitrofuran compounds, in urine and serum, using *S. faecalis* as test organism

Nitrofurantoin and other 5-nitrofuran derivatives are used extensively as antimicrobial agents, and extensive work is being conducted on new nitrofuran derivatives. This work has emphasized a need for the determination of low levels of such compounds in biological fluids. There are chemical methods (1–6) for the determination of nitrofuran compounds. However, microbiological methods are preferable for the potency determination of active compounds because these assays are more sensitive. Furthermore, compounds possessing analogous chemical structure or even the breakdown products can interfere with the chemical methods of assay, while the microbiological methods can help to assess the antimicrobial activity available.

Colorimetric and other chemical methods (1–5) involve conversion of 5-nitrofuran derivatives to 5-nitrofurfuraldehyde phenylhydrazone, followed by extraction in solvent and column chromatography or by extraction in nitromethane and color development with hyamine (6). These methods are elaborate and time consuming.

The purpose of this study was to develop a turbidimetric method for the estimation of nitrofuran compounds in biological fluids.

MATERIALS AND METHODS

Assay Medium—The medium contained 2% (w/v) glucose, 0.9% (w/v) casitone (Difco), 0.5% (w/v) yeast extract (Difco), 1.0% (w/v) sodium citrate, 0.1% (w/v) potassium phosphate monobasic, and 0.1% (w/v) potassium phosphate dibasic. The pH was adjusted to 6.8 ± 0.1. The assay medium, double strength, was prepared and sterilized at 15 lb./in.^{2} for 15 min.

Inoculation Medium—The inoculation medium was the same as the assay medium.

Indicator Strain and Inoculum—After preliminary investigation, *Streptococcus faecalis* (ATCC 10541) was selected as the test microorganism for the assay procedure. Material from the stock agar stab culture was inoculated into the inoculation medium and incubated overnight at 37°. This culture was then adjusted to a reading of 80 on a colorimeter[1] prior to inoculation into the assay medium.

Preparation of Standard Stock Solution—Stock solutions of nitrofurantoin and furazolidone were prepared by dissolving 20 mg. of each compound into 2 ml. of dimethylformamide and were preserved in the refrigerator at 4–5° in brown bottles.

Assay Procedure—The stock solutions were further diluted with sterile water to contain 10 and 5 mcg./ml. of nitrofurantoin and furazolidone, respectively. To the clean dry sterilized test tubes were added, in triplicate, 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 ml. of the standard solution, 0.5 ml. of serum or urine, and sterilized water to a total volume of 2.5 ml. Then 2.5 ml. of the inoculated double-strength assay medium was added to each tube and mixed. The tubes were suitably covered with cotton plugs and incubated at 37 ± 0.5° in a constant-temperature water bath. The incubation period was best adjudged by visual observation after 4 hr.

The control serum and urine added to the standard tubes were passed through a filter (Seitz).

Recording of Results—At the end of the incubation period, two drops of 40% formalin solution was added to each tube and thoroughly mixed to arrest further growth. Turbidity measurements were made in the colorimeter[1], using a 640 filter after adjusting to zero with proper blanks of the standard and the test samples. Averages of the standard readings were plotted against the dose on ordinary graph paper.

Statistical Treatment of Results—A statistical analysis of the assays was carried out according to the method described by Kirshbaum *et al.* (7); an analysis of variance was made and tabular and calculated F values of linearity and regression were compared to establish a dose–response relationship. Standard deviation and mean percentage were determined whenever necessary.

RESULTS AND DISCUSSION

Figures 1 and 2 present a typical dose–response curve of the test organism to nitrofurantoin in rat and human serum and urine and of furazolidone in rat serum and urine, respectively. Table I shows the analysis of variance of these data to test for the possible departure from linearity and the effect of regression. The calculated F values are significantly higher for regression and lower for linearity than those of the tabular F values. This finding shows the existence

[1] Klett–Summerson.

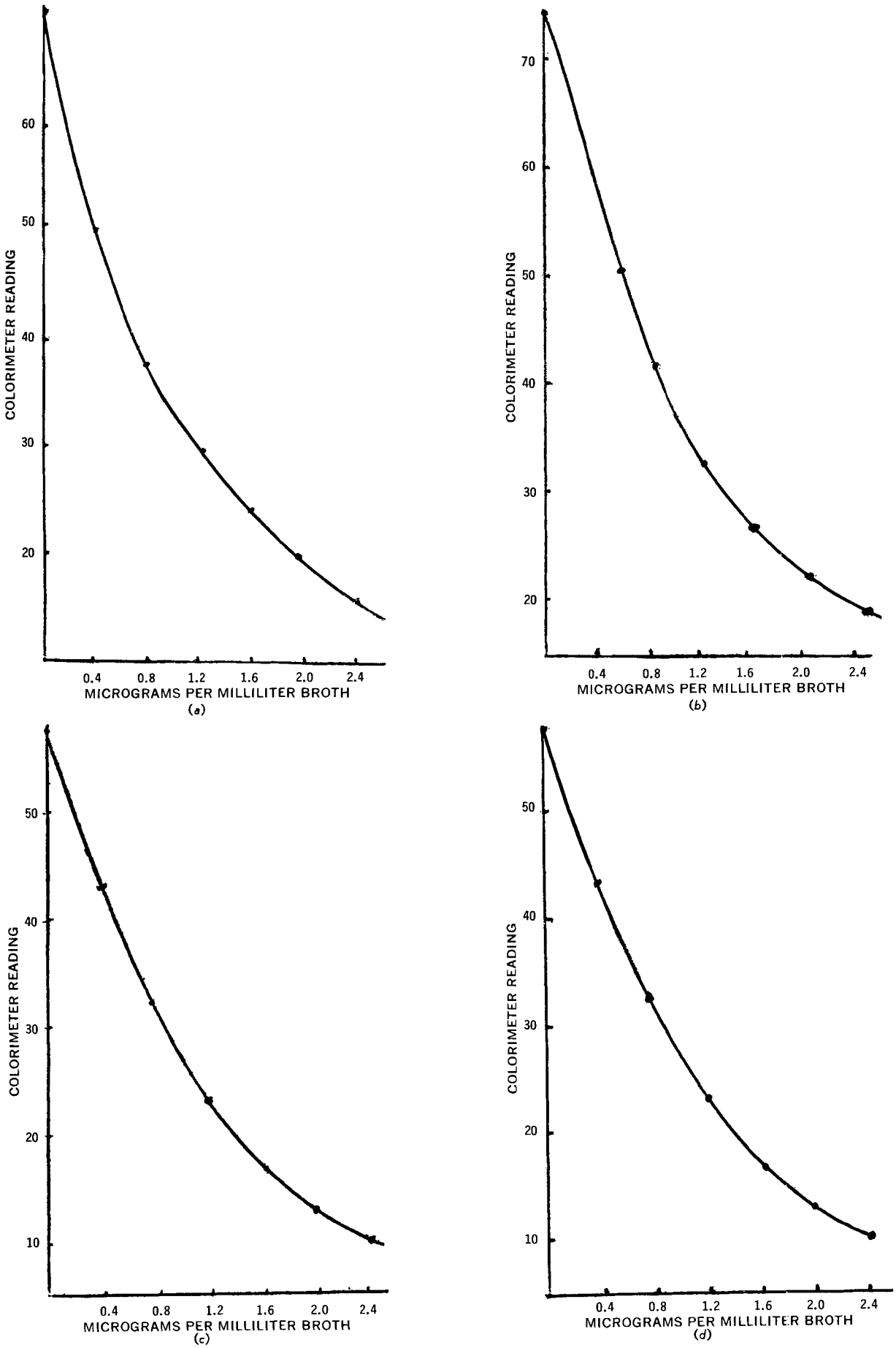

Figure 1—*Plots of assay of nitrofurantoin in:* (a) *human serum,* (b) *rat serum,* (c) *rat urine, and* (d) *human urine.*

Table I—Analysis of Variance

Compound	Assayed with	Departure from Linearity: Calculated *F*	Departure from Linearity: Tabular *F*	Regression: Calculated *F*	Regression: Tabular *F*
Nitrofurantoin	Rat serum	2.86	2.96	965.7	4.60
	Rat urine	2.84	2.96	3659	4.60
	Human serum	1.5	3.26	3543	4.75
	Human urine	2.5	3.26	3255	4.75
Furazolidone	Rat serum	0.68	3.26	331.8	4.75
	Rat urine	0.99	3.26	1506	4.75

Table II—Recovery of Nitrofurantoin and Furazolidone Added to Rat Serum

Concentration in Serum, mcg./ml.: Nitrofurantoin	Concentration in Serum, mcg./ml.: Furazolidone	Number of Determinations	Recovery: Nitrofurantoin, mcg./ml.: Mean ± *SD*	Recovery: Nitrofurantoin, mcg./ml.: Mean %	Recovery: Furazolidone, mcg./ml.: Mean ± *SD*	Recovery: Furazolidone, mcg./ml.: Mean %
2.5	2.5	6	2.61 ± 0.16	104.4	2.52 ± 0.14	100.8
5.0	5.0	6	5.1 ± 0.17	102.0	5.1 ± 0.18	102.2
10.0	10.0	5	10.01 ± 0.165	100.1	9.87 ± 0.30	98.7
15.0	15.0	6	15.07 ± 0.24	100.4	15.02 ± 0.62	100.8

of a real dose–response relationship and no departure from linearity.

Tables II and III summarize the recovery of nitrofurantoin and furazolidone added to the serum and urine in known amounts. The linearity extends over the range as indicated by the recoveries and the plots of turbidity. Samples falling near or below the lower range could be assayed with a little less accuracy.

The indicator strain selection was based on the minimum inhibitory concentration (MIC), the rate of growth, and the linearity. *Escherichia coli*, in the preliminary investigation, was found to have low MIC levels against most of the nitrofuran compounds, but all the strains tested showed a good degree of inhibition against the plain serum alone. The indicator organism, *S. faecalis* (ATCC 10541), was sensitive to most of the nitrofuran compounds and behaved in linear relationship in both serum and urine. For nitrofurantoin and furazolidone, satisfactory assays were obtained when the concentration of drug ranged from 0.4 to 2.4 mcg./ml. and from 0.2 to 1.2 mcg./ml., respectively. In these ranges the ob-

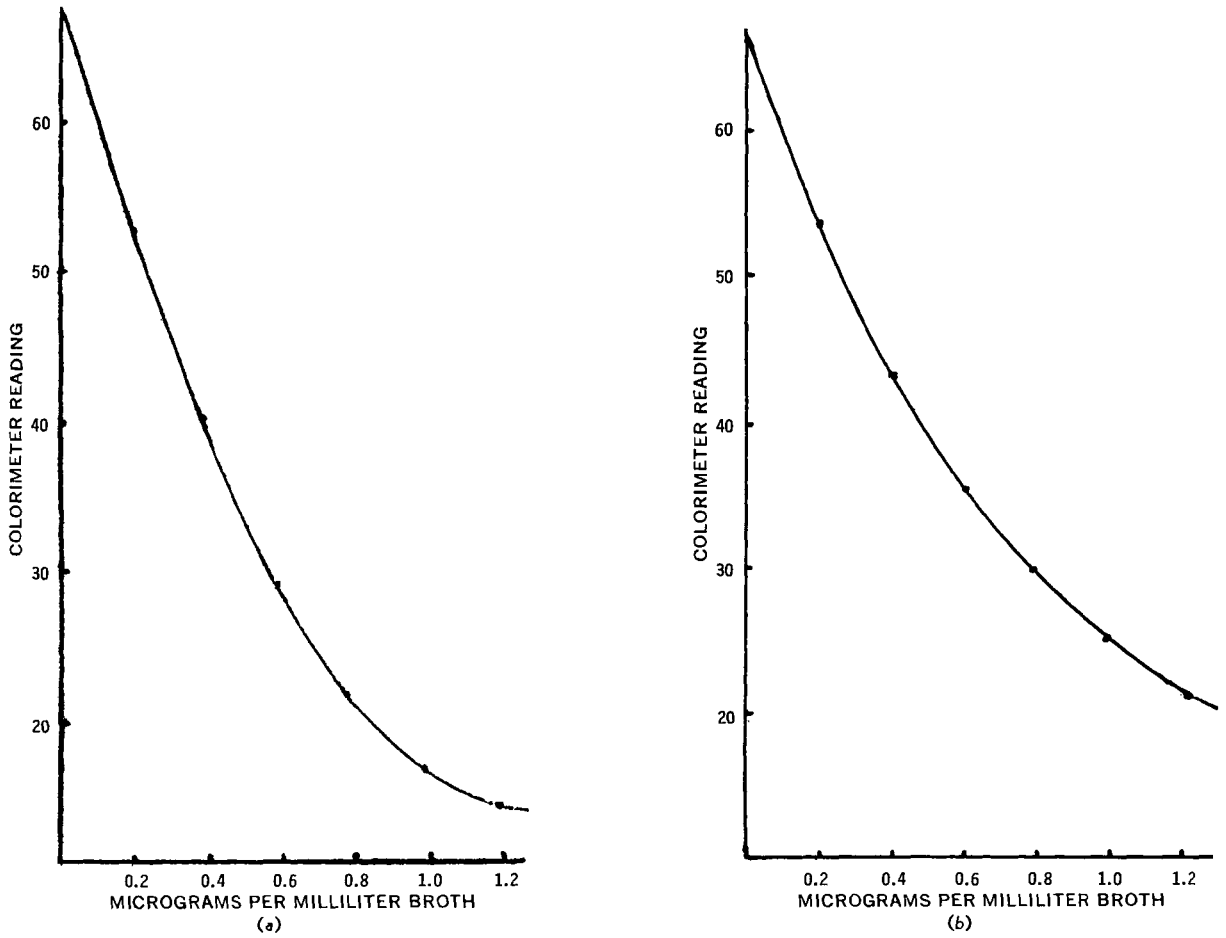

Figure 2—*Plots of assay of furazolidone in:* (a) *rat serum, and* (b) *rat urine.*

Table III—Recovery of Nitrofurantoin and Furazolidone Added to Rat Urine

Concentration in Urine, mcg./ml.		Number of Determinations	Recovery			
			Nitrofurantoin, mcg./ml.		Furazolidone, mcg./ml.	
Nitrofurantoin	Furazolidone		Mean ± *SD*	Mean %	Mean ± *SD*	Mean %
2.5	2.5	6	2.38 ± 0.39	95.2	2.53 ± 0.070	101.2
5.0	5.0	6	5.01 ± 0.18	100.2	5.11 ± 0.177	102.2
10.0	10.0	6	10.03 ± 0.37	100.3	10.05 ± 0.18	100.5
15.0	15.0	7	15.16 ± 0.60	101.0	15.06 ± 0.157	101.0

served points were a better fit to the theoretical regression and linearity.

The size of inoculum affected the growth, the form of regression, and the slope of regression in the assays with serum and urine. The optimal levels of inoculum for the assay of nitrofurantoin and furazolidone in rat and human serum and urine were 0.03 and 0.06 ml., respectively, of the standardized culture/100 ml. of the assay medium. Furthermore, the assays were influenced by the quantity of serum and urine added to the standard and the test sample tubes. Satisfactory assays were achieved when the same amount of serum and urine was present in the test and the standard. When the test samples are to be assayed at more than one level, the standard set for each level of serum or urine is to be preferred.

In turbidimetric assay, conventionally 10 ml. of the assay medium per tube is used. However, the low level of drug in serum *in vivo* and the activity of nitrofuran compounds often demand the use of larger amounts of biological fluid to fall in the linear range of the graph. In this assay, 5 ml. of the medium per tube was used, because it permits the use of a lesser volume of biological fluid for the estimation and improves sensitivity.

SUMMARY

A turbidimetric assay procedure for nitrofurantoin and its active congeners was developed using *S. faecalis*. Optimal conditions for the estimation were established using two nitrofurans (nitrofurantoin and furazolidone) in serum and urine. The method was found to be rapid and reliable for the estimation of small amounts of drug in biological fluids. The method can be equally useful and sensitive for other active congeners once the optimal conditions are determined.

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Effect of Benzene on Rat Liver Ribonucleic Acid

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Abstract □ The intraperitoneal administration of benzene to rats does not alter the sucrose density sedimentation behavior of liver ribonucleic acid but causes an increase of soluble ribonucleic acid species. Treatment of ribonucleic acid *in vitro* with benzene results in an irregular sucrose density pattern.

Keyphrases □ Benzene, intraperitoneal administration—effect on rat liver ribonucleic acid □ Ribonucleic acid, rat liver—effect of intraperitoneal administration of benzene on sucrose density sedimentation and soluble species □ Sucrose density patterns—effect of benzene on ribonucleic acid *in vitro*

Studies in this laboratory (1–3) showed that the intraperitoneal administration of benzene to rats: (*a*) causes disaggregation of liver polyribosomes which results in accumulation of ribosomal monomer-dimers and appearance of an intermediate, previously absent, peak; (*b*) affects significantly the capacity of liver polyribosomes to synthesize protein *in vitro* (>50% inhibition); and (*c*) inhibits considerably the incorporation of labeled ribonucleic acid precursors into liver polyribosomes but does not interfere with their incorporation into total liver ribonucleic acid and does not alter the size of the acid-soluble fraction radioactive label pool.

These observations suggested an action of benzene at the messenger ribonucleic acid–ribosome level which causes disorganization of the endoplasmic reticulum and breakdown of polyribosomes as is the case with liver poisons, in general (4–6). The possibility that benzene may also affect the ribonucleic acid of the ribosomes (ribosomal ribonucleic acid and r-ribonucleic