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Comparative assays of some nitrofurans in urine

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Polarographic and microbiological methods for the determination of nitrofurantoin and of *N*-(5-nitro-2-furfurylideneamino)-2-imidazoline-one (NF 246) in urine are described. In these the limit of detection is 1 μg/ml by the polarographic and 5 μg/ml by a cup plate microbiological technique. No preliminary separation of the drugs is required. The polarographic method is to be preferred, since it is a more sensitive assay and results can be obtained within an hour of receiving the sample.

THE use of nitrofurans in the treatment of urinary infections has found general clinical acceptance and the assay methods described below have been used to determine the amounts of nitrofurantoin and *N*-(5-nitro-2-furfurylideneamino)-2-imidazoline-one (NF 246) (O'Connor, Russell, Michaels, Newland & Carey, 1963) excreted in urine.

Experimental

MICROBIOLOGICAL

The cup plate assay as described for antibiotics in the B.P. (1963) was used. Nutrient agar, Oxoid CM 3, was poured in plates 12 × 12 inches and 36 cups 18 mm in diameter cut out and sealed. *Bacillus subtilis* NCTC 8236 was the test organism and spore suspensions were prepared as in B.P. (1963), page 1,105. Randomisation of experiments was achieved by means of a 6 × 6 Latin Square test design.

Nitrofurantoin assay. Dilute the urine with an equal volume of citrate phosphate buffer (pH 5·0–6·0) and pipette 1 ml into each cup.

NF 246 assay. This is in two parts: (i) to assess unchanged drug, the method is as for nitrofurantoin but using pH 6·8 buffer; (ii) for total nitrofurans as NF 246, heat urine at 70° for 60 min, cool and then treat as for the unheated sample.

Suitable standards of nitrofurantoin and NF 246 in dimethylformamide and citrate phosphate buffer were used in each test.

The plates were maintained at room temperature, protected from the light for 2 hr, then incubated at 30° for 18 hr.

Minimum inhibitory concentrations. Minimum inhibitory concentrations were measured by standard serial dilution techniques in liquid media using a 24 hr culture of *Escherichia coli* NCTC 8196 as the test organism. Nutrient broth, Oxoid CM 1, was used and the tubes incubated at 37° for 18 hr. Standards were prepared by adding the individual nitrofurans in dimethylformamide to normal male urine and proceeding as for test urines. All samples were pasteurised at 80° for 30 min.

We confirm the opinion of Carroll & Brennan (1954) that a medium of acid pH is preferable for the organisms used. The optimum pH

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range was 5·0–6·0 when testing nitrofurantoin, and 5·0–7·0 when examining NF 246. Under these pH conditions urea concentrations of up to 5% did not affect the results of the cup plate assays.

POLAROGRAPHIC

Supporting electrolyte. Dissolve Analar ammonium chloride (53·5 g) and Analar 0·88 ammonia solution (67·5 ml) in distilled water and dilute to volume (2 litres).

Standard solutions. Accurately weigh nitrofurantoin (50 mg) and dissolve in *NN*-dimethylformamide (25 ml); dilute to 100 ml with supporting electrolyte. Each ml contains 500 μg nitrofurantoin. Prepare standard solutions with supporting electrolyte to contain 25, 50, 100 and 200 μg nitrofurantoin per ml. In the same manner prepare standard dilutions in supporting electrolyte to contain 25, 50, 100 and 200 μg NF 246 per ml.

Determination of nitrofurantoin. Dilute 1 ml of urine to 25 ml with supporting electrolyte. Pipette 5 ml of this dilution into a polarographic cell containing a mercury pool anode, and maintained at 25 $\pm$ 0·5°. De-aerate the system with nitrogen (5 min) and measure the peak current of the derivative circuit at −0·32 V.* This step represents the reduction of the nitro-group (Stradins, Hillers & Jur'ev, 1959).

To 1 ml of urine add 1 ml of standard nitrofurantoin solution (25 μg/ml) and dilute with supporting electrolyte to 25 ml. Measure the peak current as above. Repeat using the standards containing 50, 100 and 200 μg/ml. Micrograms of nitrofurantoin per ml urine is given by the following formula.

$$\frac{i_d \text{ (sample)} \times \mu\text{g nitrofurantoin per ml standard solution}}{i_d \text{ (sample + standard)} - i_d \text{ (sample)}}$$

where i_d = peak current of the solution.

The determination of NF 246 is made in a similar manner.

Over the voltage range −0·1 V to −0·6 V, freshly voided urine contains no substances which interfere with this assay. For any given concentration of the nitrofuran, the diffusion current varies in urine samples from different subjects and even with the same subjects it varies with time. This fact was not appreciated by Marciszewski (1960) who also used a polarographic assay. The standard addition technique overcomes this difficulty since each sample is related to four standards.

VALIDITY OF METHODS

The validity of these methods was established by adding amounts of 25–150 μg/ml of either nitrofurantoin or NF 246 to Ringer solution, to sterilised urine and to freshly voided urine from healthy male subjects and assaying.

By the cup plate method the average drug recovery was 96·6% (s.d. 9·1).

By the polarographic assay the recovery of either drug was 102·2% (s.d. 7·5).

* A southern instrument K 1000 was used.

NITROFURANTOIN AND NF 246 IN URINE

Healthy male subjects were given doses of the drugs in accordance with the regimens outlined below. Urine was assayed by both methods. Results are shown in Figs 1 and 2 and Table 1.

TABLE 1. COMPARISON OF RESULTS OBTAINED BY POLAROGRAPHIC, CUP-PLATE AND MINIMUM INHIBITION CONCENTRATION METHODS ON A MAN TAKING NF 246

Time (hr)	μg/ml NF 246 excreted		
	Cup-plate method*	Minimum inhibition concentration	Polarographic method
08.00	100	96	112
10.00	46	48	55
12.00	92	96	87
14.00	92	96	88
16.00	244	192	270
18.00	184	192	182
20.00	348	192	338
22.00	296	192	325
32.00	164	96	181
34.00	92	48	117
36.00	50	48	63
38.00	92	48	88
40.00	276	192	244
42.00	400	384	381
44.00	138	192	138
46.00	82	96	77
48.00	146	192	138
50.00	nil	nil	1

* Heated sample.

In all cases the bladder was emptied before the start of the experiment.

Nitrofurantoin (100 mg) was administered at 07.00, 12.00, 17.00 and 22.00 hr. Urine was collected by draining the bladder at 2 hourly intervals from 07.00 to 21.00 hr; a further sample was obtained at 07.00 hr, 24 hr after administration.

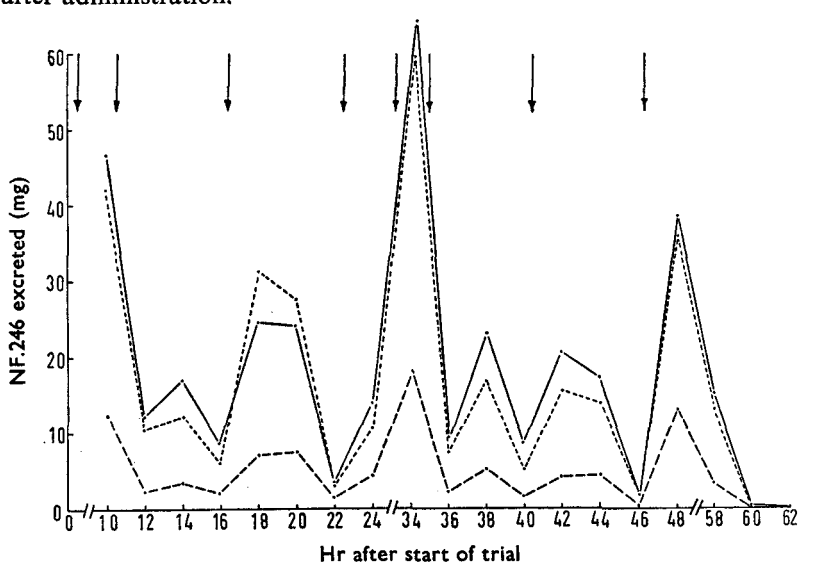

FIG. 1. Drug excretion pattern of NF 246 in urine. ——— Results of polarographic assay. - - - - - Results of heated microbiological assay. – – – – – Results of cold microbiological assays. At arrows 200 mg NF 246.

NF 246 (200 mg) was administered at 08.00, 14.00, 20.00, 22.30 hr and repeated over a second day. Urine was collected by draining the bladder at 2 hrly intervals between 08.00 and 22.00 hr.

33–49% of the nitrofurantoin administered was recovered from the urine by both methods, and 21–23% of NF 246 was recovered by a polarographic method and by a microbiological test applied to heated specimens.

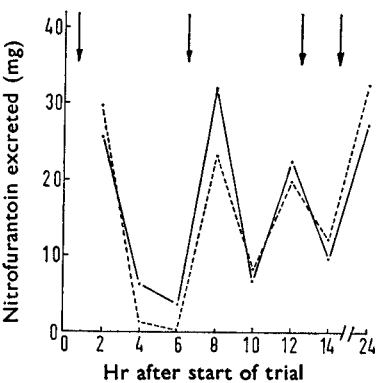

FIG. 2. Drug excretion pattern of nitrofurantoin in urine. ——— Results of polarographic assay. - - - - - Results of microbiological assay. At arrows 100 mg nitrofurantoin.

Urine from subjects receiving NF 246 showed that the polarographic and tube dilution methods gave higher results than were obtained using a simple cup plate assay. It should be noted that the tube dilution method was preceded by a sample pasteurisation, whereas for the simple cup plate assay unheated urine was used.

Pretreatment of the urine at 70° for 1 hr resulted in an increase in the *apparent* amount of drug present, and the results by cup plate assay were then in fair agreement with those obtained by the polarographic method. Urine containing nitrofurantoin does not require preliminary heating.

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