A New Method for the Determination of Nitrofurantoin in Urine

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Nitrofurantoin is presently used as an antibacterial agent for the urinary tract of both man and animals. A new, quantitative procedure for the determination of this drug in urine consists of direct extraction of the nonionized form of the drug by the use of nitromethane, addition of an alkaline reagent to the extract to produce a visible color, and determination of the nitrofurantoin concentration by spectrophotometry. The method has a sensitivity of 5 mg./L., with recoveries of 99-101% and a standard curve that is linear to 100 mg./L. Results indicate that the method measures nitrofurantoin alone, and not its metabolites, in rat, dog, and human urine.

The use of nitrofurantoin, 1-(5-nitrofururylideneamino)hydantoin, for the chemotherapy of urinary tract infections in man and animals is well established (1). Since several urologists have emphasized the correlation of clinical results with high urinary drug concentrations rather than with blood levels (2, 3), estimation of the amount of this drug in urine becomes of considerable importance. An analytical method which utilizes column chromatography to measure the concentration of this drug in urine has been reported (4). That method is a time-consuming process which is not readily conducted in the clinical laboratory. Other methods are also available (1). The present report describes a new, simplified analytical procedure for the determination of nitrofurantoin in urine. The structural formula of nitrofurantoin is shown in Fig. 1.

Materials and Methods

Reagents and Instrument

The reagents used include crystalline nitrofurantoin (Furadantin, Eaton Laboratories); 0.1N hydrochloric acid, A.C.S. (Mallinckrodt Chemical Works, Cat. No. 2612); N,N-dimethylformamide, reagent grade (Matheson Coleman & Bell, Cat. No. NX 613); and Hyamine hy-
droxide, [p-(diisobutylresoxyethoxyethyl) dimethylbenzylammonium hydroxide] 1M in methanol (443 gm./L. methanol) (Packard Instrument Company, Inc., Cat. No. 6003005). The Hyamine solution (1 ml.) is diluted to 25 ml. with absolute methanol to produce a 0.04M solution.

A Beckman spectrophotometer (Model DU) was used to measure absorbances.

**Standard Solutions**

It is necessary to protect all nitrofurantoin solutions from direct sunlight or fluorescent light at all times. Therefore all standard solutions should be stored in amber glass bottles. Exactly 50 mg. of nitrofurantoin are dissolved in 50 ml. of N,N-dimethylformamide; then 10 ml. of this solution is diluted to 100 ml. with water to produce a concentration of 100 μg./ml. This solution is then diluted with water to obtain solutions containing 5, 10, 30, and 60 μg./ml. Internally corrected standard solutions are prepared, with 1 ml. of urine, 1 ml. of the standard solution, and 3 ml. of 0.1N hydrochloric acid. The usual standard solutions are prepared, with 1 ml. of the standard solution and 4 ml. of 0.1N hydrochloric acid. When recovery experiments were performed, 0.1N hydrochloric acid and the urine used to prepare the internally corrected standard urine solutions served as control samples.

**Procedure**

To 1 ml. of urine and 4 ml. of 0.1N hydrochloric acid in a test tube, 10 ml. of nitromethane is added. The contents of the tube are mixed vigorously for 2 min. and centrifuged. Four milliliters of the nitromethane (bottom layer) are removed and transferred to another test tube. At this point, some of the samples may be cloudy in appearance. If so, the tube containing the solvent is placed under warm tap water for about 1 min. To the nitromethane extract, 0.5 ml. of the 0.04M Hyamine solution is added, and the contents are mixed and allowed to stand for at least 1 min. The concentration of the nitrofurantoin-Hyamine complex is determined by direct spectrophotometry at 400 mμ, using nitromethane, run through the procedure with 1 ml. water and 4 ml. of 0.1N HCl, as a blank. Pure nitromethane is used to set the instrument to zero absorbance. It is
recommended that the absorbance value of all samples be determined within 30 min. after the addition of the Hyamine solution (see Experimental: Stability).

A standard curve for nitrofurantoin is constructed by plotting the absorbances at 400 mμ against the amount of the drug present. The absorbance of the blank sample is subtracted from the absorbance of the unknown urine sample, and the amount of nitrofurantoin present is read directly from the standard curve. If the nitrofurantoin concentration in the urine is too high to determine the absorbance, the nitromethane extract should be diluted with additional nitromethane before the addition of the Hyamine reagent. Since the aqueous standard curve and the internally controlled urine standard curves are identical when each is corrected with its respective blank, (see under Experimental: Standard Curves), a standard curve prepared in 0.1N hydrochloric acid or standards in urine are suitable for calculating the concentration of nitrofurantoin present.

Experimental

Absorbance of Color Produced

In obtaining the visible color, Hyamine solutions of different molarities were examined, and it was shown that a solution of 0.04M was required to produce the maximum color development. The nitrofurantoin-Hyamine complex in nitromethane exhibits an absorption maximum at 400 mμ, ε = 794. Nitromethane extracts from nitrofurantoin standard solutions display an absorption maximum near 400 mμ following the addition of the Hyamine reagent (Fig. 2). Urine samples collected from subjects treated with nitrofurantoin, when subjected to the analytical method yield an absorbance maximum from 397 to 400 mμ.

Standard Curves

A standard curve for the nitrofurantoin-Hyamine complex in 0.1N hydrochloric acid is shown in Fig. 3. The drug recoveries from rat, dog, and human urine (Table 1) indicate that the standard and internally corrected standard curves are identical, and also suggest complete extraction of the nitrofurantoin into the nitromethane. The drug standard curve follows Beer's law to 100 mg./L.

Solubility

Two successive extractions in the aqueous phase established that 97% or more of the drug is removed by the initial nitromethane extraction, over a range of 5–500 mg./L. The solubility of nitrofurantoin in nitro-
methane, as determined from the nitrofurantoin-Hyamine complex, is greater than 3.7 gm./L.

**Stability**

An investigation of the stability of nitrofurantoin during the various steps in the analytical procedure revealed no appreciable loss of the drug from either 0.1N hydrochloric acid or nitromethane. When the Hyamine
reagent is added to the nitromethane solution of nitrofurantoin, this mixture yields increased absorbance of about 2% after 1 hr., and an increase of about 10% after 3 hr. A urine sample collected from a human subject treated with nitrofurantoin was used in this study. It was also shown that the 0.04M Hyamine solution in methanol was stable for a period of at least 30 days.

**Specificity of Method**

Urine samples collected from rat, dog, and human, after nitrofurantoin administration, were examined chromatographically. The drug was extracted with nitromethane and the extract spotted on paper and then treated according to a procedure reported elsewhere (5). A single yellow spot, which traveled further under acid conditions (Rf .45) than under base conditions (Rf .15), was visible under white light for each urine sample. No spots were visible with corresponding control urines. Similar Rf values were obtained with the experimental urine samples and those urine samples to which the authentic drug was added. The Rf values, both authentic and experimental, are very similar to those reported elsewhere for this nitrofuran in plasma (5). The individual yellow spots were eluted from the paper, with nitromethane, and 0.5 ml. of the 0.04M Hyamine reagent was then added to each sample. Each of the eluted spots, treated in this manner, gave an absorbance spectrum identical to that of nitrofurantoin. The above data show that this analytical procedure measures nitrofurantoin alone and not its metabolites, in rat, dog, and human urine.

**Results and Discussion**

The described method was applied to the determination of nitrofurantoin in rat, dog, and human urine following oral administration of the drug. The results are shown in Table 2.

Nitrofurantoin is a weak acid with a pKₐ of 7.2 (5). To improve the efficiency of the extraction of the drug from urine, the pH of all urine

<table>
<thead>
<tr>
<th>Species</th>
<th>Dosage</th>
<th>Form</th>
<th>Time (hr.)</th>
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<tr>
<td></td>
<td>mg./kg.</td>
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<td>10</td>
<td>Suspension</td>
<td>400</td>
</tr>
<tr>
<td>Dog</td>
<td>10</td>
<td>Tablet</td>
<td>970</td>
</tr>
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<td>Dog</td>
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<td>Capsule</td>
<td>1210</td>
</tr>
<tr>
<td>Human</td>
<td>1–2</td>
<td>Tablet*</td>
<td>145</td>
</tr>
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*One 100-mg. Furadantin tablet administered.
samples was adjusted with 0.1N hydrochloric acid to a pH lower than 5, to insure that essentially all of the nitrofurantoin present would be in the nonionized state. A pH range of 5.5–8.9 was obtained prior to the addition of the acid for the urine samples the determinations of which are shown in Table 2. After extraction of the drug, the pH of the aqueous phases of these samples ranged from 1.3 to 2.5, indicating that the nitrofurantoin present was in the nonionized form during the extraction.

Urine samples containing nitrofurantoin were analyzed by the analytical method described above and by a biological cup plate procedure reported elsewhere (6). Agreement was obtained between the results determined with the two different methods (Table 3).

Reports concerning qualitative methods for the clinical determination of nitrofurantoin in urine have appeared in the literature (7, 8). The quantitative procedure presented above is readily adaptable for this purpose. Nitrofurantoin concentrations as low as 50 mg./L. in human urine can be visually identified as a distinct yellow color in the nitromethane extract. Urine samples without nitrofurantoin do not exhibit any color.

References


