Nitrofurantoin Estimation in Urine with the Aid of Chromatography

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The determination of nitrofurantoin concentration in the urine of patients receiving Furadantin® [N-(5-nitro-2-furfurylidene)-1-aminohydantoin] for the therapy of urinary tract infections may be desirable for establishing the suitable dosage schedules. The present paper describes a chromatographic method, adapted from previous work at this laboratory (1), for separating interfering substances from Furadantin so that this compound may be determined colorimetrically or spectrophotometrically. Without some procedure for the separation and purification of the nitrofurans, spectrophotometric methods (2) applied to urine are subject to large errors because of the unavailability of true control urines. This is particularly true in the case of abnormal clinical urines where blood, excessive bile pigments, or medication may be present. The colorimetric method for Furadantin (3) cannot be used on untreated urine samples due to the presence of interfering materials. Furadantin concentrations as low as 1 or 2 mg. per 100 ml. can be determined by the chromatographic method to be described.

MATERIALS AND METHOD

Materials

1. Glassware—chromatographic columns 9 mm. (internal diameter) by 150 mm. with the bottom section connected by ground joint were used. A simple substitute of Pyrex glass tubing 9 mm. by 190 mm. may be made with three indentations near the bottom to support cotton plugs and column material. A 250-ml. filtering flask with stopper supports the

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Received for publication August 16, 1956

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column. The eluates may be collected into vials or short tubes set inside the filtering flasks.

2. Filtrol—Grade 19 from Filtrol Corporation, Los Angeles, Calif.
3. Celite—No. 545 from Johns-Manville Products Corp., Celite Division, New York, N. Y.
4. Dilute Ammonium Hydroxide—10 ml. conc. aqueous NH₄OH (28.1% NH₃) to 100 ml. with water.
5. 0.1M Trisodium phosphate.
6. 1N Hydrochloric acid.
7. 5N Hydrochloric acid.
8. Toluene—reagent grade.
9. Phenylhydrazine hydrochloride (reagent grade)—1.5 Gm. per 100 ml. of water. Prepared fresh daily and refrigerated when not in use.
10. Crystalline standard Furadantin (Eaton Laboratories).

**Procedure**

The chromatographic column is packed with a small plug of absorbent cotton followed by a layer of Celite, about 5 to 10 mm. deep. On this is packed the following Filtrol-Celite mixture to a depth of about 50 to 75 mm.: One part of Filtrol (0.5 Gm.) is added to two parts Celite (1.0 Gm.) and this mixture is made into a slurry in a beaker with water and is allowed to stand about 5 minutes for swelling. The slurry is then poured into the column while mixing and without interruption under gentle suction. The column is washed with at least 50 ml. of distilled water, with the use of reduced pressure (about 300 to 450 mm. Hg). Under these conditions a column should flow at a rate of about 4 to 6 ml. per minute. A reservoir such as a funnel connected by rubber tubing to the top of the column facilitates the operations. If a column runs too slowly (2 ml. per minute or less) it should be discarded and another one made.

The urine to be chromatographed is diluted 1:5 with water. If the diluted urine contains sediment or is cloudy, it should be centrifuged or filtered through a loose plug of cotton before it is added to the column. If a column begins to run slowly after urine has been added, stirring the top surface with a stiff wire will sometimes restore a higher flow rate. A measured volume (20 ml.) of the diluted urine sample is passed through the column. Following the urine, the column is washed successively with 20 ml. of distilled water, 5 ml. of dilute NH₄OH, and 20 ml. of water, and then the Furadantin is eluted with 0.1M Na₃PO₄ solution. The bright yellow color of the nitrofuran produced by the NH₄OH fades during the subsequent water wash. A brown urinary pigment which is not Furadantin usually is eluted by the NH₄OH wash. About 4 ml. of the first colorless
eluate is discarded and then collection is made in a small vial containing 1 ml. N HCl placed in the suction flask. The collection is continued until the eluate is no longer yellow and at least 1 ml. of uncolored solution follows. The volume of this eluate may vary from 8 to 18 ml., depending on the amount of Furadantin adsorbed on the column. During adsorption and in the subsequent steps, all Furadantin solutions should be protected from strong light, particularly from fluorescent lamps or sunlight. Eluates are usually crystal clear. If a cloudy eluate results, it is centrifuged until clear. The solution is then adjusted with HCl to pH 6.8, diluted to 20 ml., and the amount of Furadantin determined either spectrophotometrically or colorimetrically.

Spectrophotometric Determination

The absorbance of the adjusted eluate is determined at 375 mμ (the absorption maximum for Furadantin at pH 6.8) with the Beckman Model DU or other suitable spectrophotometer. With a 20-ml. sample of a 1:5 dilution of urine the concentration is calculated by the formula

\[
\text{Furadantin, mg. per 100 ml.} = \frac{\text{Absorbance} \times 5 \times 1000}{753}
\]

since the \( E_{1%}^{19} \) for Furadantin at 375 mμ is 753. If dilution of the eluate is necessary, this must be included in the calculation.

Colorimetric Determination

The colorimetric method is based on the formation of 5-nitrofurfural phenylhydrazone, its extraction and estimation (3). Three ml. of the neutralized eluate is transferred to a 18- X 150-mm. lipless test tube, followed by 1 ml. of the phenylhydrazone solution and 1 ml. of 5N HCl. After mixing, the tubes are heated 25 min. at 70° and then placed in cold water for 5 minutes. Toluene (5 ml.) is added, the tube closed with a plastic stopper\(^8\) and shaken vigorously 20-30 times. After centrifugation, the toluene layer is removed and its absorbance at 430 mμ determined. The amount of Furadantin present in the sample analyzed is read from a standard curve of the absorbances of known Furadantin solutions (i.e., 0.2, 0.4, 0.8 mg. per 100 ml.) treated in the same manner. For these readings any colorimeter capable of maximum transmission at 430 mμ and with a band width no greater than 35 mμ is suitable. A Fisher Electro-

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\(^1\) The wavelength of the absorption maximum of Furadantin varies with the pH of the solution, from 367.5 mμ at low pH values to 390 mμ at pH 9-10.

\(^8\) Polyethylene stoppers from 3-dram vials closed these tubes tightly. Stoppers with vials were purchases by catalogue No. V5225 from Scientific Glass Apparatus Co., Inc.
Fig. 1. Ultraviolet absorption curves illustrating the effect of chromatography upon a normal urine containing added Furadantin. Curve A, urine with 2 mg. per 100 ml. added drug. Curve B, urine with no added drug. Curve C, an eluate (diluted to 20 ml.) from this urine with 1 mg. per 100 ml. added drug, after chromatography. Curve D, an eluate (diluted to 40 ml.) from similar treatment, except urine contained 2 mg. per 100 ml. Curve E, an eluate (diluted to 400 ml.) from similar treatment, except urine contained 20 mg. per 100 ml. Curve F, an eluate (diluted to 20 ml.) from a similar treatment, except urine contained no drug.

photometer, a Bausch and Lomb "Spectronic-20" Colorimeter, and a Beckman Model B or Model DU Spectrophotometer have been used successfully.

The Furadantin concentration in the sample is calculated as follows:

Furadantin, mg. per 100 ml. = conc. from curve (mg. per 100 ml.) × 5

If dilution of the eluate is necessary this must be included in the calculation.

RESULTS

Direct quantitative spectrophotometric analysis of Furadantin in urines is not possible because of interference from other substances. This is illustrated by the spectrophotometric curve for a urine containing added Furadantin (2 mg. per 100 ml.) compared with the curve for the control urine (see curves A and B, respectively, Fig. 1). At this level, the presence of the drug cannot be identified by the ultraviolet absorp-

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5 The Furadantin was added as a solution in dimethylformamide (DMF) to avoid undue dilution of the urine. This solvent in the amount used (0.5 ml. with 24.5 ml. urine) had no effect on chromatography or on the subsequent analysis of Furadantin.
tion characteristics of the diluted urine. Following chromatographic treatment the eluate of the urine with added Furadantin (curve D, Fig. 1) allows ready identification as well as quantitative determination of the drug. Eluates (suitably diluted) from other levels (1 and 20 mg. per 100 ml.) of the drug added to urine and subjected to chromatographic treatment are illustrated by curves C and E. These absorption curves (C, D, and E) coincide closely with that of the pure compound in water at pH 6.8. The eluate of the control urine (curve F) contains little interfering material.

Good recoveries of Furadantin added to urine at concentrations of 0.5 to 100 mg. per 100 ml. were found by the colorimetric determination following the chromatographic procedure (Table 1). In the case of three urine samples (1, 2, and 4), 20 ml. of urine was passed through a column undiluted and the eluates were made up to 20 ml. In the case of the remaining samples (3, 5, 6, and 7) with higher concentrations, the urines were first diluted 1:5 and then 20 ml. was chromatographed. These eluates were also made to 20 ml. and, in addition to this dilution, another dilution of 1:10 was made when needed to bring the concentration of the solution for analysis in the range of the standards. The colorimetric method may not be applied directly to urine as indicated by the data of Table 1 (samples 8 and 9), unless a high concentration of Furadantin is present, so that interfering substances are reduced by dilution (sample 10).

Table 1. Recovery of Furadantin Added to Urine and Determined Colorimetrically Before and After Chromatography

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample description</th>
<th>Furadantin added (mg./100 ml.)</th>
<th>Furadantin found (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control urine, chrom.*</td>
<td>None</td>
<td>0.01 to 0.04*</td>
</tr>
<tr>
<td>2</td>
<td>Urine + drug, chrom.</td>
<td>0.5</td>
<td>0.49</td>
</tr>
<tr>
<td>3</td>
<td>Urine + drug, dil. 1:5, chrom.</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>Urine + drug, chrom.</td>
<td>2.0</td>
<td>1.3</td>
</tr>
<tr>
<td>5</td>
<td>Urine + drug, dil. 1:5, chrom.</td>
<td>20.0</td>
<td>20.7</td>
</tr>
<tr>
<td>6</td>
<td>Urine + drug, dil. 1:5, chrom.</td>
<td>50.0</td>
<td>51.1</td>
</tr>
<tr>
<td>7</td>
<td>Urine + drug, dil. 1:5, chrom.</td>
<td>100.0</td>
<td>98.2</td>
</tr>
<tr>
<td>8</td>
<td>Control urine, not chrom.</td>
<td>None</td>
<td>2.01*</td>
</tr>
<tr>
<td>9</td>
<td>Control urine, not chrom., dil. 1:6</td>
<td>1.0</td>
<td>4.16</td>
</tr>
<tr>
<td>10</td>
<td>Control urine, not chrom., dil. 1:100</td>
<td>100.0</td>
<td>108.0</td>
</tr>
</tbody>
</table>

* A 20-ml. sample of urine or its 1:5 dilution was passed through a column for each chromatographic experiment.

* This eluate was also diluted 1 to 10 before colorimetric determination.

* The total absorbance for each control urine has been estimated as Furadantin.
DISCUSSION

The solubility of Furadantin is greater in urine than in water and varies with the pH of the solution. In water at pH 4.8 the solubility is 12 mg. per 100 ml., whereas at pH 7.0 it is 19 mg. per 100 ml. Concentrations of 66 and of 90 mg. per 100 ml. have been attained by saturating urine with Furadantin at pH 7.0 and 7.3, respectively.

The concentration of Furadantin in a urine sample should be less than 20 mg. per 100 ml. before passing the sample through the chromatographic column. The preliminary 1:5 dilution has been included in the method to reduce all Furadantin concentrations to this range. Richards and coworkers (4) found urinary Furadantin concentrations of 12.5 to 50 mg. per 100 ml. in subjects receiving 100- to 200-mg. doses of the drug. In our experience (5), maximum urinary concentrations ranging from 4 to 48 mg. per 100 ml. were observed following single 100-mg. doses to normal individuals. Beutner and associates (6) reported that persons receiving 150 mg. 4 times a day exhibited maximum urinary concentrations of 20 to 46 mg. per 100 ml. For certain investigations it may be desirable to determine lower concentrations (1 mg. per 100 ml.) of the drug in urine. In such an event, the preliminary 1:5 dilution of the urine may be omitted.

In using the spectrophotometric method on chromatographed eluates, no interference was found in the presence of chloramphenicol, penicillin G, sulfisoxazole, bilirubin, blood, or blood pigments. However, interference was observed in the presence of tetracycline, chlortetracycline, or oxytetracycline. These substances apparently accompany Furadantin on the chromatographic column and exhibit ultraviolet absorption in the same range (375 m\(\mu\)).

The colorimetric method has been found satisfactory in the presence of chlortetracycline, chloramphenicol, and oxytetracycline (7). Tetracycline also does not interfere in this analytic procedure. The colorimetric method does not require a spectrophotometer for light-absorption measurements and is less subject to interference. The spectrophotometric method is somewhat quicker if a suitable instrument is available and if tetracyclines are known to be absent. Either method is satisfactory in the presence of high concentrations of Furadantin.

SUMMARY

A method for the determination of Furadantin in urine has been described. Furadantin is separated from interfering urinary pigments by a
chromatographic adsorption technic. Furadantin concentration may then be determined spectrophotometrically from the absorbance (375 μ) of the eluate, or the eluate may be further treated to form the phenylhydrazone of nitrofuraldehyde from Furadantin and the determination carried out colorimetrically.

REFERENCES

1. Eils, V. R., Unpublished data.
5. Medical Division, Eaton Laboratories. Unpublished data.