ACKNOWLEDGMENTS AND ADDRESSES

Received March 10, 1972, from the *National Research Center, Cairo, Egypt.*

Accepted for publication May 15, 1972.

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Determination of Nifurpipone in Urine

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Abstract □ Nifurpipone (5-nitrofuraldehyde-*N'*-methylpiperazinoacetylhydrazone) in urine was selectively adsorbed and eluted from cationic resin with methanolic ammonium hydroxide solution. The drug in the eluate was determined colorimetrically or spectrophotometrically.

Keyphrases □ Nifurpipone—isolation from urine, analysis □ 5-Nitrofuraldehyde-*N'*-methylpiperazinoacetylhydrazone—isolation from urine, analysis □ Ion-exchange chromatography—analysis of nifurpipone in urine

5-Nitrofuraldehyde-*N'*-methylpiperazinoacetylhydrazone (nifurpipone, I), a nitrofuran derivative, was synthesized and shown to be a useful antibacterial agent in urinary tract infections (1–3). An analytical procedure is needed to analyze this drug in the urine for studying the urinary excretion of the drug. Conklin and Hollifield (4, 5) used nitromethane to extract nitrofurantoin (or furazolidone), without extraction of its metabolite(s), from urine and alkalized the extract to produce a visible color. Chloroform was shown to be the more effective solvent for extraction of nifurpipone, but all attempts to obtain satisfactory recoveries from urines containing usual clinical amounts of the drug were unsuccessful.

The colorimetric method proposed by Buzard *et al.* (6) for determination of nitrofurans in plasma was based on the formation and colorimetric estimation of 5-nitro-2-furaldehyde phenylhydrazone. To overcome the interference in the assay of nitrofurantoin in urine, Bender *et al.* (7) placed urine samples on an activated clay[1]–diatomaceous earth[2] mixture to separate the drug from interfering pigments and then applied the colorimetric method of Buzard *et al.* (6). This technique required selected and standardized adsorption material, especially the activated clay. Furthermore, during our work the columns ran so slowly that they had to be discarded frequently.

It was found that because of the aminic characteristics of nifurpipone, it could be adsorbed on a cation-exchange resin and could be eluted from the resin with diluted aqueous methanolic ammonium hydroxide solution. The present paper describes a procedure for analysis of nifurpipone added to urine and from urine collected from subjects following a single oral administration of 100 mg. of the drug.

I

EXPERIMENTAL

Materials—Nifurpipone is a yellow powder which melts with decomposition at 167–168°. It is very soluble in methanol and chloroform; soluble in ethanol, acetone, and benzene; and insoluble in ethyl ether. Its solubility in water is 0.2%. The UV spectrum of nifurpipone in water shows two maximum peaks at 360 and 253 nm. The resin selected[3] was chromatographic grade. All other chemicals and reagents used were analytical or reagent grade.

Methods—A glass chromatographic column (20 × 1.5 cm.) was filled with 2.5 ml. of the resin and washed with 100 ml. of water.

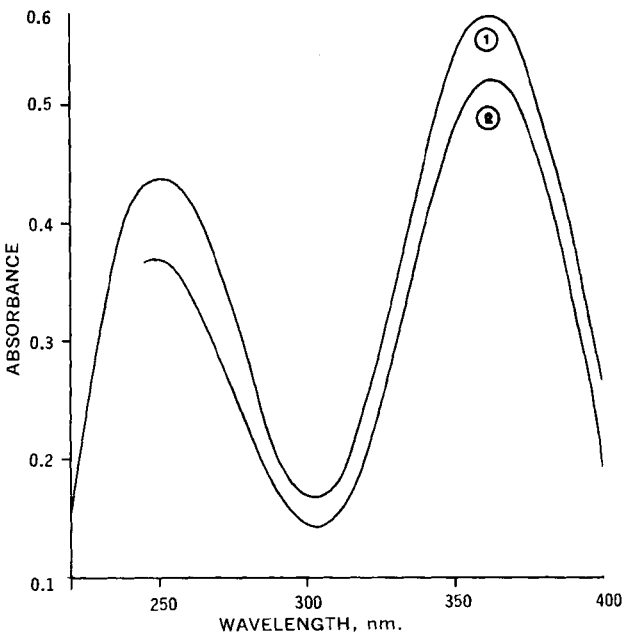

Figure 1—*Spectrophotometric curves. Key: 1, pure nifurpipone (10 mg./l.); and 2, nifurpipone from a urine sample.*

[1] Filtrol.
[2] Celite.

[3] Amberlite GC 50 (H^+), type I (100–120 mesh), Rohm and Haas Co.

Table I—Comparison of Results Obtained with Colorimetric and Spectrophotometric Analyses of the Eluates after Ion-Exchange Chromatography of Urines with Added Nifurpipone

Nifurpipone Added, mcg./ml.	Number of Assays	Recovery, mcg./ml. (Mean ± *SD*) Colorimetric Method	Spectrophotometric Method
100	10	99.70 ± 4.89	96.81 ± 3.10
		Mean of differences ± *SD* = 2.97 ± 4.95	
		t = 1.89	

Ten milliliters of urine sample, previously diluted with water to 50 ml., was passed through the resin, and the column was washed with at least 50 ml. of water. Nifurpipone was eluted with 45 ml. of 50% (v/v) aqueous methanolic ammonium hydroxide (1.5 *M*) at a flow rate of 1.5 ml./min. The eluate was collected in a 50-ml. volumetric flask, containing 3.8 ml. of glacial acetic acid for neutralization of the ammonium hydroxide, and cooled in an ice water bath. Aqueous methanol (50% v/v) was added to the column and the eluate was collected up to the mark. The eluate was filtered through Whatman No. 42 filter paper. Nifurpipone in the eluate was then determined by the following methods.

Colorimetric Method—The procedure of Buzard *et al.* (6) was used. An aliquot of the eluate was diluted with water to obtain a concentration of 2–10 mcg./ml. of nifurpipone. To 3 ml. of this solution were added 1 ml. of 5 *N* HCl and 1 ml. of 1.5% aqueous phenylhydrazine hydrochloride solution. After heating in a water bath at 70° and then cooling to room temperature, the solution was extracted with 5 ml. of toluene. The extract was filtered through anhydrous sodium sulfate, and the absorbance of the extract was read at 430 nm. The amount of nifurpipone was calculated from a standard curve.

Spectrophotometric Method—The absorbance of the eluate, which had been filtered through Whatman filter paper, was read at 360 nm.; the amount of nifurpipone was calculated using 58.2 as the absorptivity (*a*) for nifurpipone.

The blank for colorimetric and spectrophotometric methods was prepared with urine containing no nifurpipone.

RESULTS

Figure 1 shows the UV spectra obtained from a standard nifurpipone solution (10 mg./l.) and from urine with added nifurpipone and analyzed with the described procedure. As can be seen, the shapes of both curves are closely coincidental. The results obtained with the colorimetric and the spectrophotometric procedures are shown in Table I. There was no statistically significant difference between the results obtained with the two procedures. The mean recovery of nifurpipone added (from 50 to 150 mg./ml.) to urine by the ion-exchange chromatographic and spectrophotometric procedures was 97.5% (Table II). By using as the blank a urine sample collected prior to drug administration, nifurpipone from 10 urine samples collected from volunteers 2 hr. after oral administration of 100 mg. of the drug was determined with the colorimetric and spectrophotometric procedures and found to be in the range of 40.8–159.7 mcg./ml. The values obtained with the two procedures were not significantly different. The figures obtained with the microbiological assay, using *Bacillus subtilis* as the test microorganism and the USP procedure (8), were in the same range.

Table II—Recoveries from Urines with Added Nifurpipone and Analyzed with Ion-Exchange Chromatography and Spectrophotometric Assays of the Eluates

Nifurpipone Added, mcg./ml.	Number of Assays	Recovery, mcg./ml. (Mean ± *SD*)
50	5	48.12 ± 0.83
100	10	98.56 ± 2.68
150	5	144.30 ± 2.80

DISCUSSION

The resin adsorbed nifurpipone quantitatively from aqueous solution. The possible metabolite, 5-nitro-2-furaldehyde, was not adsorbed, while *N*-methylpiperazinoacetylhydrazide, another possible metabolite, was adsorbed on the resin and eluted with ammonium hydroxide solution. However, *N*-methylpiperazinoacetylhydrazide showed no absorption at 360 nm. and did not produce color by applying the method of Buzard *et al.* (6); therefore, it did not bias the result.

Nifurpipone was retained at the top of the adsorbent as a yellow color band which was not eluted with water. The color band moved to the bottom of the column during the elution with the first 10-ml. fraction of the eluent. The second 10-ml. fraction eluent was sufficient to elute all the nifurpipone from the column. Using 50 ml. of eluent in the recommended procedure gives a larger safety margin. The sensitivity of the colorimetric procedure is 5 mcg./ml. of the drug in the urine, while the sensitivity of the spectrophotometric method is 25 mcg./ml. Both of these values are lower than the therapeutically active levels and the clinically found amounts of nifurpipone in the urine (50–200 mcg./ml.).

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 3, 1971, from the *Research Division, RECORDATI s.a.s., via Civitali 1, 20128-Milan, Italy.*

Accepted for publication May 12, 1972.

▲ To whom inquiries should be directed.