ing the solution freshly each day is observed. The decomposition which occurs after several hours exposure to light is observed as a yellowing of the solution and is due to the formation of a polymer of DAN. The formation of this polymer would decrease the concentration of DAN available for reaction. In analytical work where the stoichiometric excess of DAN is large, such slight changes in concentration as would occur in 8 hr. do not affect the results.

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DRUG STANDARDS

Quantitative Determination of Furazolidone and Nifuroxime in a Water-Soluble Suppository Base

RICHARD BOICE, MURRY SEIDMAN, and B. C. SOUTHWORTH*

Abstract □ A thin-layer chromatographic procedure has been developed for the analysis of furazolidone and nifuroxime in suppositories. The sample is dissolved in a suitable solvent and chromatographed. The separated nitrofurans are then compared to respective standards densitometrically. The coefficient variation is 2.4% for furazolidone and 2.7% for nifuroxime. Evidence is presented to indicate that only the intact compounds are measured.

Keyphrases □ Furazolidone and nifuroxime suppositories—analysis □ TLC—separation □ Spectrodensitometry—TLC spot analysis □ IR spectrophotometry—identity

The present official method (1) for the determination of furazolidone and nifuroxime in a suppository base Furazolidone and Nifuroxime Suppositories (NF[1]) is hampered by the variability of the montmorillonite used for the chromatographic separation of the nitrofurans. As much as 20% of the furazolidone may be irreversibly adsorbed onto the column packing. Although this adsorption may be compensated for by running standards under identical conditions, results are somewhat variable.

Both paper chromatography (2–6, 12) and TLC (2, 7–11) have been used for the detection of furazolidone. Recently Zoni and Lauria (13) and Bortoletti and Perlotto (14) have reported the use of TLC for both the detection and the determination of furazolidone and nifuroxime in admixture. Following plate development the above authors eluted the nitrofurans from the silica gel and determined their concentrations spectrophotometrically. The relative ease with which nitrofuran derivatives may be isolated, and recent developments in instrumentation

[1] Tricofuron Vaginal Suppository, Eaton Laboratories, Division of The Norwich Pharmacal Co.

Table I—Linearity of Spot Density *Versus* Concentration

Plate No.	Furazolidone: Regression Line	Furazolidone: Correlation Coefficient, γ	Furazolidone: SD σ	Nifuroxime: Regression Line	Nifuroxime: Correlation Coefficient, γ	Nifuroxime: SD σ	Range Studied, mcg.
1	$y = 26.7 + 278x$	0.9989	4.47	$y = 21.4 + 295x$	0.9978	6.61	0.25–1.25
2	$y = -2.7 + 438x$	0.9928	7.62	$y = 7.9 + 241x$	0.9927	7.84	0.4 –0.85
3[a]	$y = 14.4 + 414x$	0.9963	6.41	$y = 15.7 + 385x$	0.9942	6.91	0.4 –0.85

[a] One furazolidone spot was rejected as more than three times the standard deviation from the regression line.

for direct plate scanning prompted the investigation of this technique for the assay of furazolidone and nifuroxime suppositories.

EXPERIMENTAL

Apparatus—Schoeffel spectrodensitometer model SD 3000, equipped with Density Computer model SDC 300, and Esterline Angus Recorder model E1101E coupled to a disk integrator.

Developing Solvent—Benzene R.–acetone R.–glacial acetic acid R. (76:20:4 by vol.).

Preparation of Standard Solution—Prepare a standard solution in acetone containing 150 mcg./ml. of NF Reference Standard furazolidone and 135 mcg./ml. of NF Reference Standard nifuroxime. Protect this solution from light.

Micropipet—Reservoir type, 5 μl. (A. H. Thomas, Cat. No. 8206-MIO).

Chromatoplates—Silica Gel G plates (E. Merck No. 5763) scored at 10-mm. intervals.

Preparation of Developing Chamber—Line the walls of the developing chamber (30 × 10 × 28 cm.) with filter paper. Add 150 ml. of the developing solvent, close the chamber tightly, and allow at least 30 min. for saturation of the chamber atmosphere. Prepare this tank daily.

Assay Procedure—Conduct this procedure in subdued light. Melt (50° maximum) and mix together the suppositories to be assayed and weigh accurately sufficient sample to contain about 15 mg. of furazolidone and about 22.5 mg. of nifuroxime (this would normally be a sample size of 6.0 g.).

Dissolve the sample in 20–30 ml. of acetone with the aid of gentle heat and transfer the solution to a 100-ml. volumetric flask. Cool the solution to room temperature, dilute it to the mark with acetone, and mix it well. Label this Solution A. Dilute 30.0 ml. of Solution A to 50 ml. with acetone. Label this Solution B. Apply 5-μl. spots of these solutions and of the standard solution to a chromatoplate as indicated in Fig. 1. Transfer the plate to the developing chamber, develop until the solvent front has proceeded 120 mm. beyond the spotting line; remove the plate from the chamber, and air-dry for 30 min. Measure the densities of the spots with the spectrodensitometer using the following parameters: wavelength, 340 mμ; monochromator slit, 1 mm.; beam divider slit, 3 mm.; density computer function, ratio, log; recorder speed, 2 in./min.

Determine the areas under the recorded curves from the integrator pen traces.

Calculations—

$$\frac{\Sigma\text{area of furazolidone in A}}{\Sigma\text{area of furazolidone in std.}} \times \frac{1.5}{\text{sample wt. (g.)}} = \%\text{ furazolidone}$$

$$\frac{\Sigma\text{area of nifuroxime in B}}{\Sigma\text{area of nifuroxime in std.}} \times \frac{2.25}{\text{sample wt. (g.)}} = \%\text{ nifuroxime}$$

RESULTS

Separation of the Actives from the Suppository Base—The R_f values for the components of this suppository are: polyethylene glycol, 0.06; polyoxyethylene palmitate, 0.08; lactic acid, 0.23; furazolidone, 0.28; nifuroxime, 0.55.

In order to ascertain that the active ingredients are not degraded in the assay procedure and to further ascertain that separation is complete, the furazolidone and the nifuroxime were eluted from the silica gel (after spotting at a heavier level than for quantitative analysis) and IR spectra recorded. The spectra compared favorably with standard spectra.

Interference from Degradation Products—Forced degradation of nifuroxime (in the suppository base *via* UV irradiation and/or thermal extremes) indicates products at R_f about 0.38 and about 0.15. Furazolidone under the same conditions shows several products at R_f 0–0.15. These products would not interfere with the above procedure. IR spectra of the eluted material from spots with R_f = 0.28 and 0.55 compared favorably with standard spectra of furazolidone and nifuroxime, respectively.

Linearity of Results—Acetone solutions containing 0.25–1.25 mcg. of furazolidone and of nifuroxime per 5 μl. of solution were prepared and treated according to the above procedure starting at "Apply 5 μl. . . ." Results are given in Table I.

The equation for calibration from the standard expected linear regression is of the form $y = a + bx$ in which y is a measure of the spot density in terms of integrator count, a is a constant corresponding to the integrator count expected from a blank, b is the slope of the regression line, and x is the amount of standard in micrograms applied to the plate.

Recovery of Furazolidone and Nifuroxime from a Suppository Mixture—A mixture of 0.250% furazolidone and 0.375% nifuroxime in a water-soluble base of polyethylene glycol, polyoxyethylene palmitate, and lactic acid was prepared to simulate a commercial vaginal suppository. Portions of the mixture were treated as indicated in *Experimental*.

Results are given in Tables II and III. Data are treated as previously (15). The average recovery of furazolidone was 99.5% with a 2.4% *SD*. The average recovery of nifuroxime was 100.2% with a 2.7% *SD*.

DISCUSSION

The results obtained in this study indicate that densitometric analysis is sufficiently accurate and precise for routine pharmaceu-

Table II—Recovery of Standard Furazolidone

Plate No.	Sample: $\bar{x}$	Sample: σ_x^2	Standard: $\bar{y}$	Standard: σ_y^2	Recovery: %	Recovery: V_T^2	Recovery: V
1	338.3	14.78	339.0	91.00	99.8	9.21	3.03
2	358.3	5.11	Rejected		—	—	—
3	302.7	69.78	308.3	23.78	98.2	10.12	3.18
4	303.0	3.00	308.3	29.11	98.3	3.39	1.84
5	321.0	6.33	316.0	25.00	101.6	3.12	1.77
6	326.3	15.44	328.7	48.78	99.3	5.96	2.44
7	328.0	4.33	329.3	17.44	99.6	2.01	1.42
					Av. 99.5%	5.64	2.4

Table III—Recovery of Standard Nifuroxime

Plate No.	Sample		Standard		Recovery		
	x	σ_x^2	$\bar{y}$	$\sigma_{\bar{y}}^2$	%	V_Γ^2	V
1	271.3	37.28	275.3	14.11	98.5	6.93	2.54
2	291.0	20.33	286.7	119.44	101.5	16.93	4.12
3	259.3	8.78	257.3	18.77	100.8	4.14	2.03
4	238.0	2.33	238.3	19.44	99.9	3.83	1.96
5	269.3	13.78	265.7	34.78	101.4	6.82	2.61
6	262.0	46.33	261.0	27.00	100.4	10.71	3.27
7	258.3	110.11	260.3	0.11	99.2	16.51	4.06
					Av. 100.2%	7.05	2.7

tical analysis. The coefficient of variation was found to be 2.4% for furazolidone and 2.7% for nifuroxime.

Some experimental factors were found to be critical in obtaining consistent and accurate results. To minimize errors due to the measurement of small volumes of solutions, a reservoir type micropipet was used. This technique, whereby the tip of the micropipet is touched to the hard surface of the chromatoplate and the liquid expelled, tended to avoid errors due to creep-back and capillation as reported by Fairbairn and Relph (16). To optimize quantitation by direct plate scanning, it is necessary to have small round spots, well isolated from interfering materials. To some degree this is dependent upon the size of the initial spot application, which should be as small and as uniform as possible (17). The particles to be measured in the spot must be sufficiently "dilute" so that all particles are illuminated and have an opportunity to attenuate the light beam. As reported by Downing (18) use of vertical lanes on the chromatoplate helps to maintain proper spot characteristics for plate scanning. Good separations were achieved with benzene–acetone mixtures and addition of acetic acid reduced tailing without affecting the separation.

Maximum absorbance of furazolidone was found to be at 365 mμ. and of nifuroxime at 330 mμ. However, 340 mμ. was the wavelength of choice to simplify the method and save analysis time; the spot density for furazolidone at 340 mμ. was about 98% of that at 365 mμ. and the spot density for nifuroxime at 340 mμ. was about 80% of that at 330 mμ. A linear relationship was found to exist for furazolidone and nifuroxime at 340 mμ., between the spot density and spot concentration, covering a range of 0.25–1.25 mg. Specificity is ensured by recording of the curves at the R_f's for the drug constituents. Quantitative evaluation was achieved by use of a double beam scanning spectrodensitometer without elution of the drugs from the adsorbent.

By use of multiple spots of sample and of standard and by averaging of results, a procedure is obtained which is sufficiently accurate for stability studies and sufficiently rapid and precise for routine control.

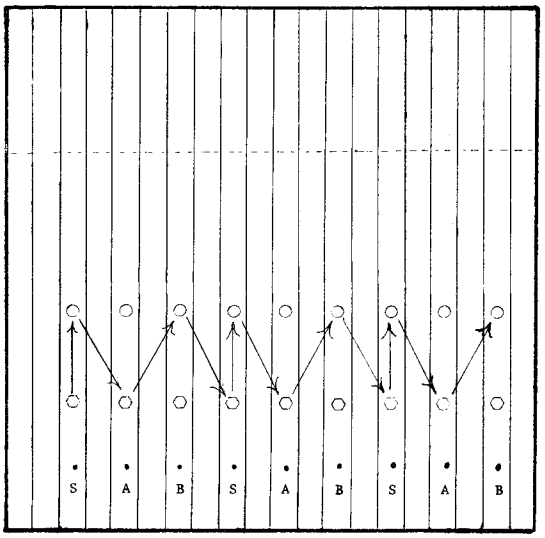

Figure 1—*Typical chromatoplate. Key: S, standard solution; - - -, solvent front; A, solution A; B, solution B;* ○, *nifuroxime;* ⬡, *furazolidone;* →, *order in which spots are read.*

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