Separation and Quantitative Assay of Furazolidone and Nifuroxime in Suppositories by Magnesium Silicate Adsorption Column Chromatography

PAMELA F. WOJTOWICZ and JOSEPH J. DILIBERTO*

Abstract □ A rapid and quantitative column chromatographic separation method, in which a patented activated magnesium silicate is used as the adsorbent, is presented for the analysis of a suppository containing furazolidone and nifuroxime. Nifuroxime is eluted from the column with chloroform, and furazolidone is eluted with 5% glacial acetic acid in dimethylformamide. The separated nitrofurans are then compared spectrophotometrically to respective standards.

Keyphrases □ Suppositories, furazolidone–nifuroxime—separation, analysis □ Furazolidone–nifuroxime suppositories—separation, analysis □ Nifuroxime–furazolidone suppositories—separation, analysis □ Column chromatography—separation, furazolidone–nifuroxime suppositories □ UV spectrophotometry—analysis, furazolidone–nifuroxime suppositories

The current official assay procedure (1) for furazolidone and nifuroxime in furazolidone and nifuroxime suppositories NF[1] involves the column chromatographic separation of the nitrofurans, using a mixture of an acid-activated montmorillite[2] and a purified siliceous earth[3] as the adsorbent, followed by UV spectrophotometric quantitation. In this procedure, the variability of the montmorillite presents several problems (2). It is difficult to obtain the suggested elution rate of 0.5 ml./min., even when vacuum is applied. The assay time is critical because furazolidone and nifuroxime undergo degradation through hydrolysis and exposure to light. When vacuum is applied, fine particles of adsorbent accompany the active ingredient effluents and cause high background interference, making quantitation difficult, particularly for furazolidone. Reportedly, up to 20% of the furazolidone may be irreversibly adsorbed onto the column packing.

Methods for the detection and quantitation of furazolidone and nifuroxime, utilizing TLC, were reported. Zoni and Lauria (3) and Bortoletti and Perlotto (4) eluted the separated nitrofurans from a developed silica gel plate and determined their concentrations spectrophotometrically. Boice *et al.* (2) determined the concentrations of furazolidone and nifuroxime on a developed plate with a recording densitometer.

The need for a rapid, accurate, and reliable method for determining furazolidone and nifuroxime in combination, without the use of highly specialized equipment, prompted this investigation.

EXPERIMENTAL

Apparatus—The following were used: UV-visible spectrophotometer; and glass column, 250 mm. long by 10 mm. i.d., fitted with a fritted-glass disk and a Teflon stopcock.

Solvents—Chloroform, methanol, and dimethylformamide, all spectral grade, were used.

Adsorbent—Patented activated magnesium silicate[4] was used.

Acid–Dimethylformamide Solution—Dilute 10 ml. of reagent grade glacial acetic acid to 200 ml. with dimethylformamide.

Standard Solutions—Dissolve NF reference standard furazolidone, 5 mcg./ml., in the acid–dimethylformamide solution; dissolve NF reference standard nifuroxime, 7.5 mcg./ml., in methanol–chloroform (1:9).

Sample Preparation—Dilute a sample equivalent to approximately 37.5 mg. of nifuroxime to 500 ml. with methanol.

Chromatographic Column Preparation—Transfer 5.0 g. of the adsorbent to a chromatographic column, and tap gently to settle the adsorbent. Insert a small glass wool plug above the adsorbent.

Caution: Protect chromatographic column, standard solutions, and all sample solutions from light.

Assay Procedure—Place a 50-ml. volumetric flask under the column, and pipet a 5.0-ml. aliquot of the sample preparation onto the column. Allow the solution to pass into the glass wool, and wash the inner wall of the column with a little chloroform. Allow this chloroform wash to pass into the column, and then elute with 50 ml. of chloroform at a rate of 1–2 drops/sec. Discontinue the elution, wash the column tip, and dilute to volume with chloroform. Reserve this eluate for the quantitative determination of nifuroxime.

Add the acid–dimethylformamide solution to the column, and collect 5 ml. of the column eluate in a graduated cylinder. Replace the graduated cylinder with a 50-ml. volumetric flask, and continue the elution with the dimethylformamide solution until approximately 48 ml. has been collected[5]. Dilute to volume with acid–dimethylformamide, and reserve for the quantitative determination of furazolidone.

Quantification—Protect eluted solutions from light; read absorbances within 4 hr., using 1-cm. quartz cells.

Nifuroxime—Record the UV absorbances of the reference standard and sample solutions from 430 nm. with methanol–chloroform (1:9) as the reference solution, reading the absorbance maximum at 338 nm.

Furazolidone—Record the UV absorbances of the reference standard and sample solutions from 470 nm. with the acid–dimethylformamide solution as the reference, reading the absorbance maximum at 370 nm.

Calculate the percentages of furazolidone and nifuroxime present in the sample.

[1] Tricofuron is a registered trademark of Eaton Laboratories, Division of Norwich Pharmacal Co., Norwich, N. Y.
[2] Filtrol Grade No. 19 supplied by Filtrol Corp., Los Angeles, Calif.
[3] Celite 545, supplied by Johns-Manville, New York, N. Y.
[4] Florisil, 60–100 mesh, Fisher Chemical Co., Catalog No. F-100.
[5] The exact volume of eluting solvents is not critical. Elution patterns indicate that 100% of the nifuroxime present is recovered from the column in the first 10 ml. of chloroform and 100% of the furazolidone in the first 25 ml. of acid–dimethylformamide.

Table I—Recovery Data for Mixtures of Nifuroxime and Furazolidone

Analysis Number	Milligrams Recovered—Nifuroxime	Milligrams Recovered—Furazolidone
Recoveries for Synthetic Mixture		
1	36.3	25.2
2	36.8	25.0
3	36.5	25.2
4	36.5	25.1
5	36.3	24.9
6	36.4	24.9
7	36.3	25.1
8	36.4	24.8
9	36.2	25.0
10	36.3	25.0
Milligrams added	37.5	25.6
Milligrams recovered ± *SD*	36.4 ± 0.2	25.0 ± 0.1
Percent recovered ± *SD*	97.1 ± 0.4	97.7 ± 0.6
Recoveries for Drugs Added to Commercial Product		
1	18.6	12.3
2	18.8	12.1
3	18.7	12.0
4	18.6	11.7
5	18.7	12.3
Milligrams added	19.2	12.2
Milligrams recovered ± *SD*	18.7 ± 0.1	12.1 ± 0.2
Percent recovered ± *SD*	97.3 ± 0.4	99.0 ± 1.6

RESULTS AND DISCUSSION

The accuracy of the proposed method is based upon the results of 10 replicate analyses of a synthetic solution, containing 37.5 mg. of nifuroxime and 25.6 mg. of furazolidone in 500 ml. of methanol, and the results of five replicate determinations of a previously analyzed commercial preparation, with 19.2 mg. of nifuroxime and 12.2 mg. of furazolidone added to the sample solution. Better than 97% of each ingredient was recovered. The results of these analyses and statistical treatment of the data are presented in Table I.

Quintuplicate assays were performed on four lots of a commercial product, declared to contain 0.375% nifuroxime and 0.25% furazolidone. The results of these determinations and the statistical treatment of the data are presented in Table II. The replicate results from the recovery analyses and from the product assays were used to determine statistically the precision of the data.

In a limited study, the product was exposed to heat and UV radiation to force degradation of the active ingredients. In the original assay, the nifuroxime concentration was 0.367 and 0.337% after degradation. Furazolidone was quantified at 0.239% originally and 0.141% after degradation.

No change in peak shape or shift in absorbance maxima was noted in the spectra of degraded sample ingredients. This indicates that the column procedure presented is capable of distinguishing between active ingredients and degradation products. The higher degree of furazolidone decomposition indicates that its instability probably accounts for the lower original assay results in the commercial product.

Table II—Analysis of Commercial Samples of Nifuroxime and Furazolidone[a]

Sample	Analysis Number	Percent Found—Nifuroxime	Percent Found—Furazolidone
A	1	0.365	0.237
	2	0.367	0.238
	3	0.368	0.239
	4	0.368	0.240
	5	0.368	0.240
	Percent ingredient ± *SD*	0.367 ± 0.001	0.239 ± 0.001
	Percent declaration ± *SD*	97.9 ± 0.3	95.6 ± 0.5
B	1	0.352	0.233
	2	0.352	0.236
	3	0.354	0.233
	4	0.356	0.231
	5	0.355	0.230
	Percent ingredient ± *SD*	0.354 ± 0.002	0.233 ± 0.002
	Percent declaration ± *SD*	94.4 ± 0.5	93.0 ± 0.9
C	1	0.362	0.233
	2	0.364	0.233
	3	0.362	0.231
	4	0.363	0.233
	5	0.365	0.231
	Percent ingredient ± *SD*	0.363 ± 0.001	0.232 ± 0.001
	Percent declaration ± *SD*	96.8 ± 0.4	92.9 ± 0.4
D	1	0.362	0.237
	2	0.363	0.234
	3	0.365	0.235
	4	0.365	0.233
	5	0.364	0.235
	Percent ingredient ± *SD*	0.364 ± 0.001	0.235 ± 0.002
	Percent declaration ± *SD*	97.0 ± 0.3	93.9 ± 0.6

[a] Declared to contain 0.375% nifuroxime and 0.25% furazolidone.

The results of this study indicate that an activated magnesium silicate adsorbent may be used successfully to separate nifuroxime and furazolidone quantitatively. The column chromatographic procedure is sufficiently accurate and precise for routine pharmaceutical analysis.

REFERENCES

(1) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 324.

(2) R. Boice, M. Seidman, and B. C. Southworth, *J. Pharm. Sci.*, **58**, 1527(1969).

(3) G. Zoni and E. Lauria, *Boll. Chim. Farm.*, **106** (10), 706 (1967); through *Chem. Abstr.*, **68**, 43214u(1968).

(4) B. Bortoletti and T. Perlotto, *Farmaco, Ed. Prat.*, **23**, (7), 371(1968); through *Chem. Abstr.*, **69**, 56891h(1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 9, 1971, from the *Food and Drug Administration, Buffalo, NY 14202*

Accepted for publication April 30, 1971.

*Present address: DL Skin Care Product, Inc., Buffalo, NY 1420