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A stability-indicating assay for nitrofurazone by paper chromatography

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Summary

A simple paper chromatographic method for separation and quantitative determination of nitrofurazone in the presence of its degradation products was developed. The method was based on impregnation of the chromatographic paper with pH 4.0 buffer containing 10% w/v urea. The percentage recovery of nitrofurazone after chromatography suggested that the accuracy of the prescribed method is adequate for its application in pharmaceutical preparations. The method was applied for the determination of nitrofurazone content in surfactant, polyethylene glycol and aqueous solutions with or without ultraviolet light absorbers present.

Introduction

Nitrofurazone is used as a topical antibacterial agent and the commercial preparations available are a cream, an ointment and a solution usually of 0.2% w/v strength in a polyethylene glycol formulation. The various official compendia state that the drug formulations should be stored in light-resistant containers and exposure to direct sunlight and excessive heat should be avoided. However, there is a paucity of published data on the stability and potential photolability of the drug.

Official methods of assay involve the use of spectrophotometric methods (BP, 1988; USP, 1990), although dosage forms such as nitrofurazone ointment and topical solution necessitate pretreatment using column chromatography for removing interfering materials (USP, 1990). Several methods currently available for the analysis of nitrofurazone range from colorimetry (Rao et al., 1979), spectrophotometry (Agrawal and Patel, 1986; Tamer and Omar, 1989), and fluorometry (Taniguchi et al., 1973), through gas chromatography (Ryan et al., 1975) to high-performance liquid chromatography (HPLC) (Sugden et al., 1983). However, in none of these methods has the simultaneous determination of intact drug in the presence of its decomposition products been reported.

Separation of intact drug from its photodecomposition products by paper chromatography

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(Wunderlich, 1958; Shahjahan, 1979) and identification of photolysis products on a reverse-phase HPLC column (Quilliam et al., 1987) have been reported. To date, the quantitative separation of nitrofurazone from its degradation products by chromatography or other methods has not been reported. By using the paper chromatographic method developed here the quantitative separation of nitrofurazone from its degradation products has been achieved.

Although an ascending paper chromatographic method (Shahjahan, 1979) using *n*-butanol : acetic acid : water (4 : 1 : 5) as the solvent system with Whatman No. 4 paper separated nitrofurazone from its decomposition products in simple aqueous solution, this procedure was not successful with formulations containing surfactant or polyethylene glycols. Hays and Grady (1971) found that urea precipitated cetyl alcohol and polyethylene glycol from nitrofurazone cream as urea inclusion compounds. Urea solution was also successfully employed as an immobile phase for paper chromatographic determination of oxytetracycline (Sina et al., 1971). Based on these findings, the use of urea solution as an immobile phase in a paper chromatographic method appeared to offer a promising approach which would enable separation and subsequent determination of nitrofurazone in the presence of surfactants and polyethylene glycols. Paper impregnated with urea could not be used with the *n*-butanol : acetic acid : water (4 : 1 : 5) solvent system as the acid affected the impregnated urea.

The purpose of the present study is to establish an accurate procedure for the determination of nitrofurazone in different pharmaceutical preparations in the presence of its decomposition products.

Materials and Methods

Materials

Nitrofurazone (Human Grade; Batch No. 6B 5017; m.p. 220–224°C with decomposition) was obtained from Smith Kline and French Laboratories Ltd (Herts, U.K.). Uvinul D-50 was purchased from GAF (Great Britain) Ltd. The surfactants used were partially purified samples of Texofors (ABM Chemicals Ltd, U.K.). All solvents and laboratory reagents were of 'Analar' grade (BDH, U.K.). Double-distilled water was prepared using an all-glass distillation unit (QVF, Stoke-on-Trent).

Evaluation of optimum chromatographic conditions

Two starting lines 5 cm in length were marked out, leaving a 5 cm gap between them on a sheet of Whatman No. 4 chromatographic paper (28 cm × 23 cm) about 3 cm from one end of the sheet. The lines were at least 3 cm from the edges of the paper. A series of these papers was impregnated with McIlvaine's pH 5.0 buffer containing 10% w/v urea. Another series of papers was impregnated with McIlvaine's buffer of pH values between 2.2 and 8.0, also containing 10% w/v urea. A third set of papers was impregnated with pH 4.0 buffer containing different concentrations of urea up to 40% w/v. The papers were then blotted between sheets of absorbent paper and spotted with the appropriate solutions.

A range of solvent systems saturated with urea was used with the urea-impregnated papers. Solvents without urea presaturation were used for the development of urea-free papers of pH 4.0.

To ensure that efficient separation of nitrofurazone could be achieved from both solutions containing polyethylene glycol and the surfactant solutions, the following procedure was employed.

Spotting solutions for chromatography were prepared by dilution of either 0.1 M surfactant or 0.25 M polyethylene glycol 1000 solutions containing nitrofurazone (2×10^{-3} mol l^{-1}). 0.1 cm^3 of the diluted solution was spotted along the 5 cm length of the starting line. The chromatograms were then partially dried in air and, while still damp, subjected to ascending development along the machine direction of paper in chromatography jars containing 100 cm^3 of the solvent system. When the solvent front reached about 20 cm above the starting line, the papers were removed, dried in air and the separated spots or bands identified under short-wavelength ultraviolet light. The chromatography jars were covered with

aluminium foil to protect the chromatograms from light.

Validation of assay procedure

A 0.2 cm^3 quantity of a freshly prepared water : ethanol (1 : 2) solution containing 0.25 mg cm^{-3} of nitrofurazone was spotted along a 15 cm horizontal line on Whatman No. 4 chromatographic paper, previously impregnated with 10% w/v urea in McIlvaine's pH 4.0 buffer. Several of these chromatograms were then partially dried in air and, while still damp, were transferred to chromatographic jars containing ethyl acetate : *n*-butanol : benzene (3 : 2 : 1) saturated with urea. When the solvent front reached about 20 cm above the starting line, the chromatograms were removed and dried in air (about 1 h). A blank paper without nitrofurazone was similarly treated. The yellow horizontal area of nitrofurazone was excised from the chromatogram and divided into squares of approx. 5 mm dimension. An equivalent area was removed from the blank paper. The subdivided areas from each chromatogram were extracted with 10 cm^3 of water : ethanol (1 : 2) mixture by shaking for 1 min in a glass vial (30 cm^3 capacity) and then being allowed to stand for another 1 min. The eluate was then centrifuged at 4000 rpm for 10 min (Gallenkamp Junior centrifuge). The concentration of nitrofurazone present in the eluate was determined spectrophotometrically by recording the absorbance at 372 nm and refering to a standard calibration curve constructed by dissolving known concentrations of nitrofurazone in the same solvent.

A series of chromatographic determinations was conducted to calculate the percentage recovery of nitrofurazone.

Determination of nitrofurazone in different formulations

The method described above was applied in the determination of nitrofurazone content in surfactant, polyethylene glycol and aqueous solutions with or without ultraviolet light absorbers present. The formulations of surfactant or polyethylene glycol contained 0.4 mg cm^{-3} of nitrofurazone and were diluted with twice their volume of water. Subsequently, 0.2 cm^3 of the diluted sample was applied directly onto the chromatographic paper, and the assay was completed as described previously. Calibration curves were constructed for each formulation of polyethylene glycol, surfactant and aqueous solution by spotting 0.2 cm^3 of varying concentrations of nitrofurazone onto chromatographs, carrying out the chromatographic procedure as described and determining the absorbance of the eluate.

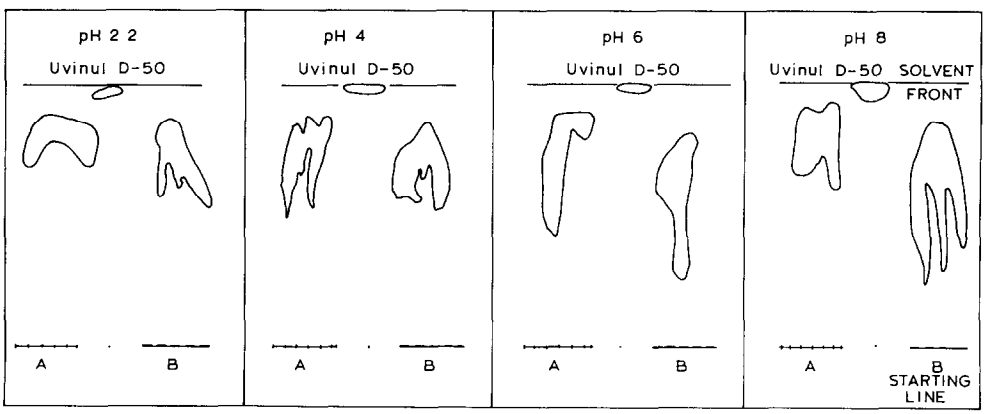

Fig. 1. Effect of pH on the chromatographic behaviour of nitrofurazone and Uvinul D-50 in different formulations. (A) Polyethylene glycol formulations; (B) surfactant formulations. (Chromatographic paper) Whatman No. 4, 10% w/v urea impregnated; (solvent system) ethyl acetate : *n*-butanol : benzene (3 : 2 : 1).

Results and Discussion

Assay validation

The results obtained in impregnating chromatographic papers with pH 5.0 buffer containing 10% w/v urea and using the different solvent systems for development demonstrated that ethyl acetate : *n*-butanol : benzene (3 : 2 : 1) is by far the best solvent system for these formulations.

Fig. 1 shows the chromatograms obtained after impregnation of the paper with 10% w/v urea in buffers of different pH values (pH 2.2–8) upon development in the ethyl acetate : *n*-butanol : benzene (3 : 2 : 1) system. Table 1 lists the areas of

TABLE 1

Effect of pH on the area of the nitrofurazone spot and R_f values of the drug and Uvinul D-50

10% w/v urea impregnated at pH	Polyethylene glycol formulation		Surfactant formulation		R_f of Uvinul D-50
	Area (cm^2)	R_f of nitrofurazone	Area (cm^2)	R_f of nitrofurazone	
2.2	13.81	0.82	13.69	0.74	0.97
4	14.23	0.70	15.41	0.69	0.97
6	14.93	0.65	16.81	0.54	0.98
8	17.88	0.77	28.96	0.58	0.95

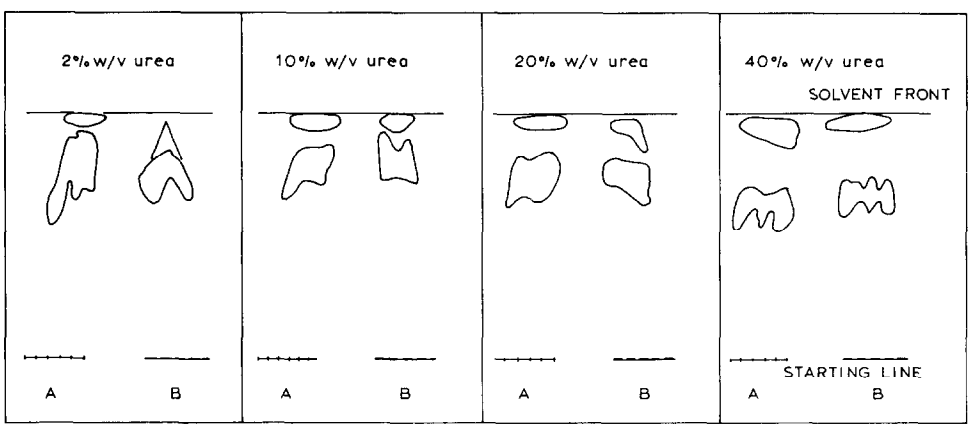

Fig. 2. Effect of urea concentration on the chromatographic behaviour of nitrofurazone and Uvinul D-50 in different formulations. (A) Polyethylene glycol formulations; (B) surfactant formulations; (▬▬) nitrofurazone; (———) Uvinul D-50. (Chromatographic paper) Whatman No. 4, pH 4.0 buffer impregnated; (solvent system) ethyl acetate : *n*-butanol : benzene (3 : 2 : 1).

TABLE 2

Effect of urea concentration on the area of the nitrofurazone spots and R_f values of drug and Uvinul D-50

% w/v urea at pH 4.0 buffer	Polyethylene glycol formulation		Surfactant formulation		R_f of Uvinul D-50
	Area (cm^2)	R_f of nitrofurazone	Area (cm^2)	R_f of nitrofurazone	
0	22.45	0.53	20.27	0.56	–
2	15.70	0.80	10.67	0.75	0.90
10	11.76	0.78	9.58	0.81	0.96
20	12.56	0.74	9.94	0.71	0.92
40	12.94	0.63	9.87	0.67	0.95

intact drug spots and the R_f values for intact drug and Uvinul D-50 in chromatograms impregnated with buffers of different pH.

On comparison of the R_f values of nitrofurazone and Uvinul D-50 and the areas of nitrofurazone spots at different pH levels, it appears that impregnation with pH 2.2 buffer yields the best separation conditions. However, there is evidence in the literature (Spross, 1953) that the stability of the drug is poor at such a low pH. For this reason, impregnation of the chromatographic paper with pH 4.0 buffer containing 10% w/v urea was considered to be the best compromise between effective separation of the drug and the potential problem of instability during chromatographic separation.

Fig. 2 shows diagrams of chromatograms impregnated with pH 4.0 buffer containing different concentrations of urea. Table 2 shows the nitrofurazone spot areas as well as the R_f values of drug and Uvinul D-50 under these chromatographic conditions.

It is evident that impregnation of the chromatographic paper with 10% w/v urea in McIlvaine's pH 4.0 buffer is the most suitable for the determination of nitrofurazone in different formulations.

The effect of urea can be explained on the basis that it increased the solubility of nitrofurazone whilst precipitating the surfactant and polyethylene glycol near the starting line of the chromatogram. Urea did not form a complex with the drug nor did it interfere with the subsequent ultraviolet assay of the eluted spot (Shahjahan, 1978).

Experiments in which the chromatogram containing 10% w/v urea at pH 4.0 buffer was developed along the machine direction of the paper and also in the reverse direction revealed that development in the reverse direction lowered the R_f of all the compounds and prolonged the developing time. Therefore, in this work, development was always carried out in the machine direction of the chromatographic paper.

Fig. 3 shows a chromatogram obtained for a light-degraded nitrofurazone solution using the above optimum conditions. It can be seen that the nitrofurazone spot is clearly separated from the spots of the decomposition products. When the nitrofurazone spot was eluted with dimethylformamide and the eluate respotted on Whatman No. SG 81 paper, with development in the descending direction with the organic phase of the *n*-butanol : acetic acid : water (4 : 1 : 5) system, it still produced a single spot which was identical to that produced by a pure nitrofurazone sample (Shahjahan, 1979). The absence of degradation spots on rechromatography of the eluate indicated that nitrofurazone had not undergone decomposition during the development procedure.

TABLE 3

Percentage recovery of nitrofurazone after chromatography

Number of experiment	Amount of nitrofurazone in 0.2 cm^3 of spotting solution (μg)	Amount of nitrofurazone found after chromatography (μg)	Percentage recovery	Mean percentage recovery	Standard deviation
1	50.0	49.8	99.6		
2	50.0	47.7	95.4		
3	50.0	48.6	97.2		
4	50.0	47.4	94.8	97.3	2.5
5	50.0	49.5	99.0		
6	50.0	50.5	101.0		
7	50.0	48.0	96.0		
8	50.0	46.7	93.4		
9	50.0	49.9	99.8		
10	50.0	48.5	97.0		

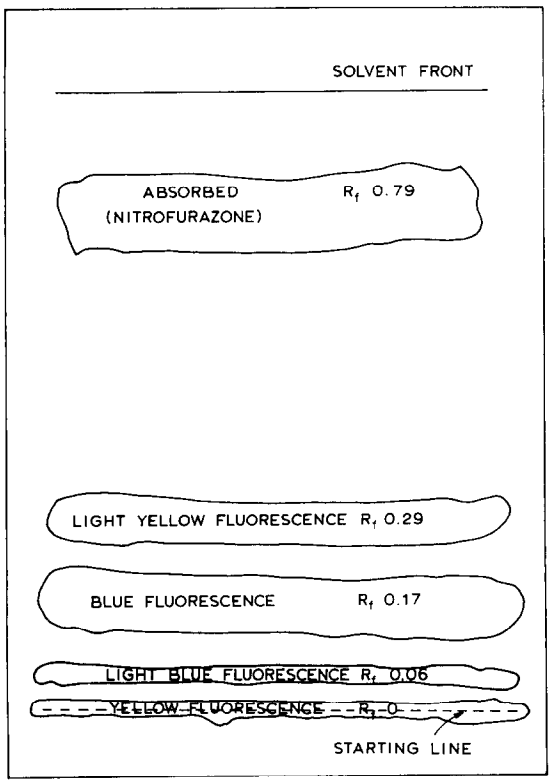

Fig. 3. Chromatogram of degraded nitrofurazone solution on paper impregnated with pH 4.0 buffer containing 10% w/v urea. (Chromatographic paper) Whatman No. 4; (solvent system) ethyl acetate : *n*-butanol : benzene (3 : 2 : 1).

Analysis in different formulations

The percentage recovery of nitrofurazone from the chromatogram as shown in Table 3 suggests that the accuracy of quantitative determination of the drug using the prescribed method is adequate for its application in pharmaceutical preparations.

The results of comparing the calibration curves constructed from different formulations are listed in Table 4.

These calibration curves were compared using Student's *t*-test, and no significant differences could be observed between them at the $p = 0.01$ level.

Since it was observed that, although the calibration curves did not vary with the formulations used, minor differences occurred due to the composition of the solvent system and the quality of the chromatographic paper due to variation between batches, it was decided to run a reference chromatogram spotted with a known concentration of nitrofurazone on every occasion where quantitative evaluation was carried out.

It was found that, in order to achieve good separation, the drug sample should be diluted 2-fold with water and spotted on a horizontal line. This was necessary to reduce loading of the ultraviolet light absorbers and the surfactant or polyethylene glycols. In order to achieve sufficient sensitivity, it was necessary to have at least 10 μg of nitrofurazone spotted on the horizontal line, so that the final concentration in the water: ethanol eluate was of the order of 1 $\mu g\ cm^{-3}$.

In conclusion, the paper chromatographic method described above is ideally suited for the analysis of nitrofurazone in the presence of its

TABLE 4

Comparison of the calibration curves obtained for different formulations

Amount of nitrofurazone in 0.2 cm^3 of spotting solution (μg)	Absorbance values for eluted nitrofurazone solution [a]		
	Aqueous formulations	Polyethylene glycol formulations	Surfactant formulation
10	0.073	0.073	0.075
20	0.149	0.152	0.160
30	0.221	0.238	0.232
40	0.317	0.313	0.311
50	0.400	0.399	0.385

[a] Mean of 3 determinations, using water : ethanol (1 : 2) mixture as solvent and 1 cm path length silica cells.

decomposition products. It is precise and stability-indicating, offering distinct advantages over the compendial spectrophotometric assay.

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