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INVESTIGATION OF A METHOD OF ANALYSIS AND THE DECOMPOSITION PRODUCTS OF FURAZOLIDONE

Contract Report to the Department of the Environment
Furazolidone is an antimicrobial drug which is used for therapeutic and prophylactic purposes in cattle, pigs and poultry. It is said to decompose rapidly on exposure to light. It is considered important to be able to quantify residues of the drug and its decomposition products in water because of the possible risks to health.

A method for the determination of furazolidone in animal tissues using high performance liquid chromatography (HPLC) has been adopted by MAFF. The work reported here was to ascertain whether that method could be adapted for water analysis and to investigate the photodegradation of the drug.

Furazolidone was separated from water using cartridges containing octadecylsilane (ODS) phases, which were back-extracted with methanol prior to HPLC analysis. The limit of detection under the analytical conditions employed was estimated to be 5 ng. This is equivalent to 1 µg/l for a sample volume of 1 litre.

Although ultraviolet spectrophotometry indicated the eventual total photodecomposition of furazolidone, HPLC analysis of a photodecomposed solution revealed four decomposition products present in only relatively small amounts. Mass spectrometry and infra-red spectroscopy confirmed that furazolidone remained predominantly unchanged.

The findings are surprising and cannot be explained at present.
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REFERENCES
1. INTRODUCTION

Furazolidone [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone] is an antimicrobial agent. It is reported to be used for the treatment of bacterial diarrhoea and gastroenteritis and also in veterinary medicine for therapeutic and prophylactic purposes in cattle, pigs and poultry. It is said to exhibit mutagenic and carcinogenic properties and it is therefore considered important to be able to quantify its residues in, among other media, water.

Its solubility in water is low and it is said to decompose rapidly on exposure to light.

Methods for the determination of furazolidone in animal tissues using HPLC have been published and a method has been adopted by MAFF(1-3).

The work reported here, carried out for the Department of the Environment was to ascertain whether the MAFF method could be adapted to water analysis, to estimate the limit of detection, and to investigate the photodegradation of furazolidone.
2. PROGRAMME OF WORK

(i) In the dark, to prepare an aqueous solution of furazolidone and examine it by the MAFF HPLC method omitting the steps used to extract it from flesh and to estimate the limit of detection of the method.

(ii) To expose a portion of the aqueous solution to sunlight for 15 minutes and to ultra-violet light for 24 hours and to re-examine the solution by HPLC to estimate the degree of decomposition.

(iii) To use HPLC to separate the decomposition products from (ii) above and attempt to identify them using mass spectrometry or other means.
3. SOLUTION OF FURAZOLIDONE

Furazolidone has only a limited solubility in water, and it was found difficult to prepare standard aqueous solutions with confidence. Stock solutions used for calibration purposes were made up with the aid of methanol. The solutions used for photodecomposition studies were prepared at nominal concentrations in water only. It was considered important not to risk any interference that organic solvents might have on the course of decomposition and that it was not so important that the substance might not be fully dissolved in carrying out a qualitative study.

All solutions of furazolidone were stored in a refrigerator in flasks wrapped in aluminium foil to exclude light. These precautions prevented significant deterioration.
4. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FURAZOLIDONE

4.1. Method

The HPLC technique employed closely followed the MAFF method(3).

Liquid chromatograph : Varian LC50

Column : 2 Zorbax Reliance ODS-5 columns in series

Eluting solvent : 30% methanol, 70% 0.1M acetate buffer (0.05M acetic acid + 0.05M sodium acetate)

Solvent flow rate : 1 ml/min (back pressure of 190 atmospheres at 30°C)

Detector : Varian UV5 variable wavelength spectrophotometric detector, at 255-266 nm linked to a chart recorder and a Hewlett-Packard 3390A integrator

Samples were injected via a 10 μl Rheodyne loop.

A calibration curve for furazolidone was prepared from dilutions of a 25 mg/l stock solution.

4.2. Results

Chromatograms were as shown in Figure 1. The calibration curve is shown in Figure 2. Two plots are shown, one of integrated peak areas, the other of measured peak heights. Both are linear over the range 10-250 ng, applied to the column in 10 μl injections.

The reproducibility of loop injection for a 10 μl sample of the 25 mg/l standard (13 injections over a period of 4 days) was determined. For integrated peak area measurements the coefficient of variation was 0.98%, and for measured peak heights was 3.92%.

Using the chromatography conditions described, the limit of detection on the basis of a signal to noise ratio of 2:1 is about 5 ng of furazolidone.
5. EXTRACTION OF FURAZOLIDONE FROM AQUEOUS SOLUTION

Vroomen et al\(^{(2)}\) concentrated the drug from plasma and urine using a Merck Extrelut 1 column and eluting with 6 ml ethyl acetate. In this work various octadecylsilane (ODS) phases were studied.

5.1. SEP-PAK C18 cartridges (Waters Associates)

Cartridges were first washed with 10 ml acetone, 10 ml methanol (HPLC grade) and 20 ml distilled water. Metered volumes of a range of aqueous furazolidone solutions were forced through the cartridges using large volume (up to 100 ml) gas-tight syringes. Cartridges were then back flushed with 2 ml methanol into screw cap vials ready for analysis by HPLC. Sampling was carried out rapidly in a darkened room to minimise photodecomposition, and extracts were stored wrapped in aluminium foil.

5.2. Bondelut C18 cartridges (Analytichem International)

These cartridges were treated in the same way as the SEP-PAK cartridges.

5.3. CO-PELL C18 precolumns (Reeve Angel)

These are used for on-column sampling with connection to the HPLC system via a 6-port, 2-position switching valve. Using this technique the whole of the sample extract is backflushed on to the analytical column and, potentially, sample volumes as low as 50 ml will give detection limits of about 0.1 \(\mu g/l\). Sampling takes longer however, because the flow rate is limited to 5 ml/minute.

5.4. Results

It was found that whereas SEP-PAK cartridges were effective in extracting furazolidone from solution, both the Bondelut and CO-PELL columns showed no capacity for the drug. This was a surprising result which cannot be explained at present. All further tests on the separation of furazolidone were carried out using SEP-PAK cartridges.
5.5. Effect of buffering on extractability

5.5.1. Experimental

Extractions were carried out at different concentrations of the acetate buffer used in the HPLC eluting solvent: 0.05M, 0.1M and 0.2M.

5.5.2. Results

No noticeable variation in peak height was obtained and it was inferred that buffering has no effect on extraction efficiency.

5.6. Effect of variation in furazolidone concentration at fixed sample volume

5.6.1. Experimental

50 ml aliquots of furazolidone solutions at concentrations of 10, 20, 50, 100 and 500 µg/l were extracted using SEP-PAK C18 cartridges as described above, and the methanolic extracts analysed by HPLC.

5.6.2. Results

Both integrated peak areas and measured peak heights obtained from the range of volumes extracted are shown plotted in Figure 3. Breakthrough begins at around 10 µg of applied furazolidone. For the linear portions of the curves, the extraction efficiency is about 94%, by comparison with the standard curves for loop injection of furazolidone (Figure 2).

5.7. Effect of variation of sample volume at a fixed concentration of furazolidone

5.7.1. Experimental

25, 50, 100 and 200 ml volumes of a 250 µg/l standard were extracted and analysed as above.

5.7.2. Results

Figure 4 shows the plots of peak areas and peak heights against the mass of furazolidone extracted. Breakthrough occurs at about 12 µg. Extraction efficiency is about 83%.
6. PHOTODEGRADATION OF FURAZOLIDONE

6.1. Experimental

Portions of a 7 mg/l aqueous solution were exposed to diffuse daylight and to ultra-violet radiation. This work was carried out in November/December when the sky was almost continuously overcast and of course at the shortest daylight period of the year. Daylight exposures were constrained to about 3 hours.

The ultra-violet source used was an Allen A409 long wave range lamp. UV exposures were extended to 9 hours.

Decomposition was followed by spectral scans during the exposures using a Cecil CE599 scanning spectrophotometer across the wavelength range 200-550 nm.

A further test was carried out with a 25 mg/l solution exposed to UV radiation for a total of 70 hours. The absorbance spectrum was obtained and the degraded solution was analysed by HPLC by the procedure already described but monitoring at 3 wavelengths, 366 nm, 260 nm and 240 nm. The degraded solution was also freeze dried, preparatory to mass spectrometric and infra-red examination.

Since furazolidone is more soluble in methanol and acetone than in water, solutions at higher concentration in these solvents were also exposed to UV light, and the products obtained after evaporation of the solvent also examined by MS and IR.

Attempts were made to separate the products by gas chromatography (GC).

6.2. Results

The effects of daylight exposure are shown in Figure 5. Furazolidone initially exhibits absorbance maxima at 260 nm and 360 nm. During the exposure there is an initial rapid decrease in the absorbances at both these wavelengths with the formation of a band at 230 nm. As time progressed the band at 230 nm itself decreased, giving way to a more diffuse absorbance across the range 200-240 nm, and the appearance of another weak diffuse absorbance at about 420-460 nm.

The effects of UV exposure are shown in Figure 6. They are generally similar to those of daylight.
exposure except that the decrease in the absorbances at 260 nm and 360 nm are not as rapid as in daylight.

The changes in absorbances at 260 nm and 360 nm are shown plotted in Figures 7 and 8.

Figure 9 shows the spectral scan of the 25 mg/l solution exposed to UV radiation for 70 hours. The absorbance at 360 nm is not in evidence but there is a high absorbance at 200-280 nm.

HPLC of the degraded solution (Figure 10) shows four degradation products. They are not completely resolved but are well separated from furazolidone. They elute earlier than the parent substance suggesting that they are more polar. The products do not appear on the chromatogram monitored at 366 nm. The peak on the chromatogram monitored at 366 nm has a retention time identical with furazolidone which is surprising since the absorbance band was greatly reduced in the spectrum in Figure 9. Its measured peak height corresponded with a concentration of 25 mg/l, no change from the original. The product peaks which feature on the chromatograms monitored at 240 nm and 260 nm are absent from chromatograms of freshly prepared furazolidone solutions monitored at those wavelengths.

The visible sign of the photodecomposition was a change in colour of the solution from yellow to orange. There was no visible evidence of the formation of any insoluble polymer. In methanol and in acetone the colour changes were more intense, from yellow through orange and red to dark brown.

Neither furazolidone nor any detectable decomposition product passed through the gas chromatograph and therefore GCMS was not possible. Instead solid probe MS was done on the parent solid and the solid extracts following exposure to UV. The mass spectra of the parent furazolidone and of the photoexposed extracts were all almost identical, not sufficiently distinguishable for any attempt to be made at identifying any products.

Likewise the infra-red spectra were also practically identical.
The MAFF method of analysis was developed to determine furazolidone residues in tissues, and employed extraction with ethyl acetate. That particular technique is not applicable to water analysis. Column extractions from liquid media have been reported\(^2\) and that approach was adopted here, using ODS cartridges. Why good recovery was possible from certain ODS brands but not from others is very surprising and cannot be explained at present. The implication is that it will be necessary to specify the range of ODS cartridges which can be used for this particular analysis.

The HPLC conditions closely followed those in the MAFF method except that a 10 µl injection loop was used not a 100 µl loop. The limit of detection of furazolidone was estimated at 5 ng, which compares with 2 ng as found by MAFF. If it were possible to extract furazolidone from a litre volume of sample, this would give a detection limit of 1 µg/l.

UV spectrophotometry indicated an almost total change in furazolidone on prolonged exposure to UV light. The change is more rapid even in dull daylight than under a UV lamp. However while HPLC revealed the presence of four decomposition products, the concentration of the parent furazolidone was virtually unchanged. Both mass spectra and IR spectra of the photoexposed material were little different from those of the parent material.

These findings are surprising and imply that decomposition is readily reversible and occurs through tautomerisation, isomerism, polymerisation or such like process. It is also possible that the change is due to autoxidation and not photodecomposition.

Clearly further work is required if the findings of this work are to be elucidated.
REFERENCES


3. MAFF. Furazolidone analysis. Private communication.
Figure 1. Liquid Chromatogram of Furazolidone
Figure 2. HPLC Calibration of Furazolidone 10ml Injections of Standard Solutions
Figure 3. Extraction of Furazolidone from Water Using SEP-PAK Cartridges
Fixed Sample Volume (50ml)
Figure 4. Extraction of Furazolidone for Water Using SEP-PAK Cartridges.
Fixed Concentration of Furazolidone (250 μg/l)

Peak height (mm)

PEAK HEIGHT

INTEGRATED AREA

Integrated peak area

150K
100K
50K

10 μg
20 μg
30 μg
40 μg
50 μg
60 μg
Figure 5. Degradation of Furazolidone on Exposure to Daylight

Absorbance

Wavelength (nm)

0 MINUTES

30 MINUTES

200 MINUTES
Figure 6. Degradation of Furazolidone on Exposure to Ultraviolet Radiation

Absorbance

Wavelength (nm)

0 MINUTES

10 MINUTES

560 MINUTES
Figure 7. Photodegradation of Furazolidone Change in Absorbance at 360nm

Absorbance (360 nm)

Exposure time (mins)

DAYLIGHT

UV RADIATION
Figure 8. Photodegradation of Furazolidone Change in Absorbance at 260 nm

Absorbance (260 nm)

Exposure time (mins)

DIFFUSE DAYLIGHT

UV RADIATION
Figure 9. Absorbance Spectrum of Furazolidone after Exposure to UV Light for 70 hours
Figure 10. HPLC of Photodegraded Furazolidone

Monitoring at 240 nm

I = Injection
F = Furazolidone

Monitoring at 260 nm

Monitoring at 366 nm