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Note

High-performance liquid chromatographic analysis of furazolidone in liver and kidney

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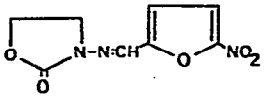
Furazolidone, 3-(5-nitrofurfurylideneamino)-2-oxo-oxazolidine, is a 3-nitrofuran used as an antibacterial agent against livestock diseases. Most nitrofurans are known to be mutagenic and carcinogenic[1–5], and our laboratory has therefore developed a method for the detection of residues of this antibiotic in pig liver and kidney.



FURAZOLIDONE

Cieri[6] described a high-performance liquid chromatographic (HPLC) method for determining residues of furazolidone in feeds. A direct HPLC method for the routine analysis of pig liver and kidney is described here.

EXPERIMENTAL

Chemicals and reagents

Furazolidone (Aesculaap, Boxtel, The Netherlands) was dissolved in acetonitrile; dilution with acetonitrile gave standard solutions containing 10–100 ng of furazolidone per 20 μl. All of the reagents were of analytical-reagent grade. Acetonitrile (Nanograde, Mallinckrodt, St. Louis, MO, U.S.A.) and distilled water, used as eluents in liquid chromatography, were pre-filtered through a G-1 glass filter.

Apparatus

A Hewlett-Packard Model 1084B liquid chromatograph equipped with a variable-wavelength UV detector (Hewlett-Packard Model 79875A) was used. A pre-column (stainless steel, 100 × 2.1 mm I.D.) was packed with Perisorb RP-8 (30–40 μm) and the analytical column (stainless steel, 250 × 4.6 mm I.D.) was packed with Hypersyl SAS. The mobile phase was water–acetonitrile (75:25) at a flow-rate of 1.75 ml/min; the temperature was ambient and the detection wavelength was 360 nm.

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Extraction

Minced samples were extracted by macerating 50 g of sample with 200 ml of ethyl acetate and 50 g of anhydrous sodium sulphate in an Ultra Turrax at moderate speed. The macerate was centrifuged for 5 min at 2500 *g* and the extract was collected. A 100-ml volume of the extract was evaporated to dryness by means of a rotary vacuum evaporator. The residue was dissolved in 2 ml of acetonitrile, warmed to 40°C and immediately cooled to remove fat particles. The solution was filtered through a G-2 glass filter.

Partition clean-up (optional)

A 100-ml volume of the extract was evaporated to dryness by means of a rotary vacuum evaporator. The residue was transferred into a separating funnel, using *ca.* 20 ml of *n*-hexane, and extracted twice with 50 ml of acetonitrile (saturated with *n*-hexane) shaking the funnel well for 3 min each time. The combined acetonitrile phases were evaporated to dryness and the residue was dissolved in 2 ml of acetonitrile. The solution was warmed to 40°C immediately cooled to remove fat and filtered through a G-2 glass filter.

High-performance liquid chromatography

A 20-μl volume of the solution was injected into the liquid chromatograph. Standard solutions containing 10–100 ng of furazolidone per 20 μl were also injected.

RESULTS AND DISCUSSION

Chromatograms of blank and treated samples are shown in Figs. 1 and 2. The partition step had to be used for samples containing more than 2% fat. For routine or screening work the optional clean-up procedure can be omitted.

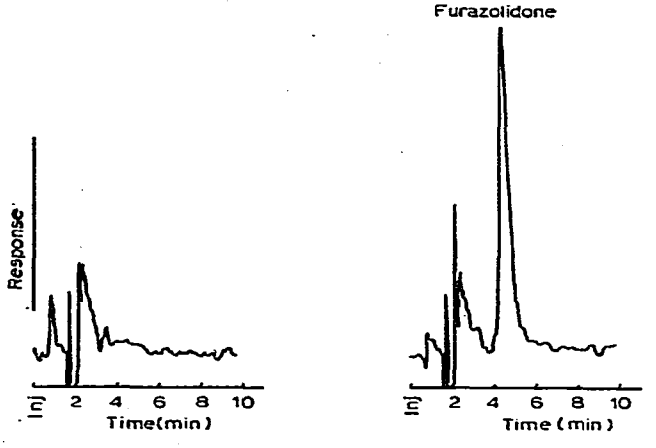

Fig. 1. Chromatogram of extract from blank pig liver after partition. Column, 250 × 4.6 mm I.D., Hypersyl SAS; pre-column, 100 × 2.1 mm I.D., Persisorb RP-8 (30–40 μm); mobile phase, water–acetonitrile (75:25); flow-rate, 1.75 ml/min; temperature, ambient; injection volume, 20 μl; UV detection at 360 nm.

Fig. 2. Chromatogram after partition of blank pig liver treated with furazolidone (0.42 ppm). Conditions as in Fig. 1.

The recovery of furazolidone was determined by analysing pig liver and kidney samples spiked with furazolidone at levels from 0.12 to 1.0 mg/kg. The results are shown in Table I.

TABLE I

RECOVERIES AFTER CLEAN-UP BY PARTITION OF FURAZOLIDONE FROM BLANK PIG LIVER AND KIDNEY TREATED WITH FURAZOLIDONE STANDARD SOLUTIONS

Average values are given for three recovery experiments each carried out with four concentrations of furazolidone.

Furazolidone added (ppm)	*Average recovery (%)*	
	Pig liver	*Pig kidney*
0.12	92.8	92.2
0.25	98.2	96.5
0.33	97.6	96.8
1.0	96.2	89.3

The limit of detection was *ca.* 0.05 mg/kg and a concentration of 0.08 mg/kg gave a peak height of 10% full-scale deflection at a detector sensitivity of 0.1 a.u.f.s.

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