CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Furazolidone in Swine Plasma

Purpose
Determination of furazolidone in swine plasma.

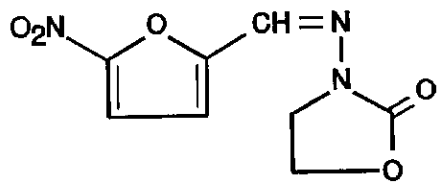

Figure 1. Structure of furazolidone.

Furazolidone (F1, 3-[[(5-nitro-2-furanyl)methylene]-amino]-2-oxazolidinone) is an antibacterial and antiprotozoal agent belonging to the 5-nitrofuran group of antibiotics. It is used both for the treatment of human urinary tract infections and as a supplement to livestock feeds [1]. Therapeutic concentrations in humans average 2 µg/mL blood. Sensitive assay procedures are needed both for therapeutic monitoring and for the detection of residues in animal products intended for human consumption.

Existing Methods
Colorimetry and microbiological assay, which fail to discriminate among related nitrofurans, GC and LC.

Conditions
System: BAS 200 Problem Solver equipped with EC and UV detectors
Electrode: BAS Glassy Carbon
Potential: - 0.75 V vs Ag/AgCl
Wavelength: 362 nm
Column: 3 µm, C 18 reverse-phase, 100 x 3.2 mm (PN MF-6213)
Mobile Phase: 90% (v:v) 15 mM dibasic sodium phosphate, 15 mM sodium citrate, pH 4.0, 10% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: EC: 125 pg injected standard, 1.1 ng/mL plasma UV: 175 ng injected standard, 3 ng/mL plasma (all at S/N = 3).
Linear Range: EC and UV: 0.5-100 ng injected standards, 10-100 ng/mL plasma.

Sample Preparation
1. Wash Bond-Elute® C-18 solid-phase extraction columns with 1 mL methanol followed by 1 mL water.

2. Combine 1 mL swine plasma and standards as appropriate and load onto each column.

3. Wash each column with 3 1-mL washes of 0.05 M sodium phosphate pH 7.0.

4. Elute the samples with 1 mL 30% aqueous methanol.

5. Dry the eluates in a vacuum desiccator or in a stream of nitrogen. Redissolve in 200 µL mobile phase, deoxygenate (see below) and inject 50 µL into the chromatograph.

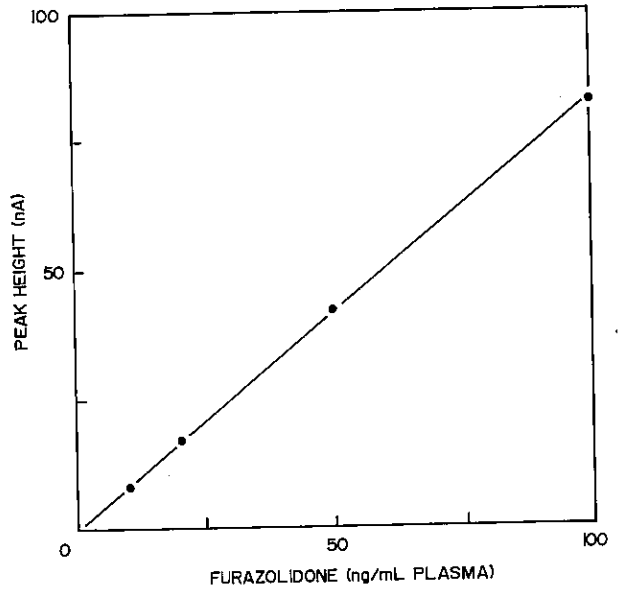

Figure 2. Calibration curve for spiked swine plasma samples.

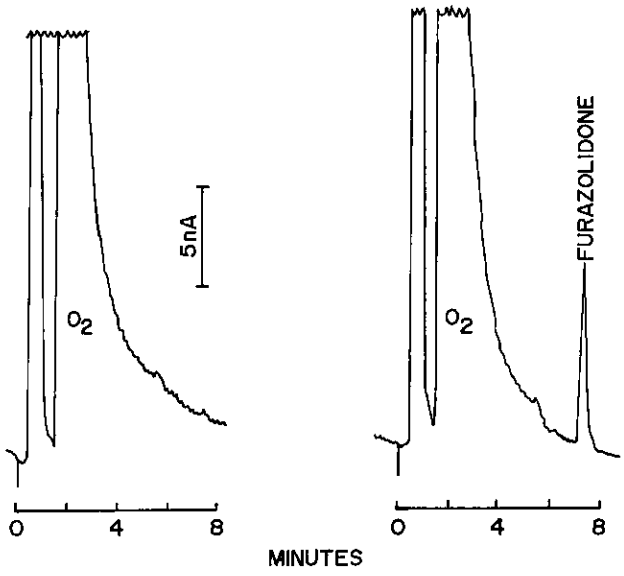

Figure 3. Chromatograms of blank (left) and spiked (10 ng furazolidone/mL) plasma. The large tailing peak beginning at 1 1/2 minutes is oxygen.

Notes

A calibration curve for spiked plasma samples is presented in F2, and sample chromatograms are presented in F3.

Oxygen is reducible at the negative potential used here, and must be removed from both the mobile phase (where it produces a high background) and the sample (where it produces an interfering peak). Removal from the mobile phase is easily accomplished on the BAS 200, which has built-in helium sparging capability and stainless steel plumbing.

Partial removal of oxygen from samples was accomplished by a 2-minute bubbling with helium saturated with mobile phase immediately prior to injection. More rigorous deoxygenation can be accomplished by passing helium through the syringe while it sits in the injection valve [2].

The mobile phase was deliberately adjusted so that furazolidone eluted late, thus minimizing EC interference by oxygen. Late elution is not necessary for UV detection alone, since oxygen is not UV active. Moreover, early elution produces higher peaks and therefore lower detection limits. Increase the acetonitrile concentration of the mobile phase to 15-20% for early elution.

References

1. Veale, H.S. and G.W. Harrington, J. Chromatogr. 240 (1982): 230-234.

2. Lloyd, J.B.F., J. Chromatogr. 256 (1983): 323.