Journal of Chromatography, 240 (1982) 230–234
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,683

Note

Determination of furazolidone in swine plasma using liquid chromatography

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First received December 2nd, 1981; revised manuscript received December 29th, 1981)

Furazolidone is used as a therapeutic antibacterial agent in swine, cattle, and poultry and is also used as a growth promotant in swine. In the latter use the drug is added to feeds in subtherapeutic doses for most of the life of the animal. The Food and Drug Administration recently proposed to list furazolidone as a category III drug for medicated feeds[1]. This action would continue the present "zero" tolerance level (no residue) restriction for this drug in edible tissue because of the concern over the potential carcinogenicity of this compound.

In connection with other studies, we required a sensitive method for the quantitative determination of furazolidone in swine plasma. Plasma levels of furazolidone have been determined by a colorimetric method[2]. In this method furazolidone is hydrolyzed to 5-nitro-2-furaldehyde. This compound is subsequently reacted with phenylhydrazine to yield the corresponding phenylhydrazone which is determined by measurement of the absorbance at 430 nm. This method is nonspecific and is also relatively insensitive. The lowest level of furazolidone reported was 1 μg/ml.

Methods have recently been reported which used liquid chromatography (LC) for the determination of furazolidone in turkey[3,4] and chicken[5] tissue and in animal feeds[6–10] but not in plasma. We report a sensitive method for the determination of furazolidone in swine plasma using LC in which both ultraviolet (UV) and electrochemical detection were examined.

EXPERIMENTAL

Reagents and materials

Furazolidone [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidin-2-one] was obtained from Norwich-Eaton (Norwich, NY, U.S.A.). Glass-distilled methanol (Burdick and Jackson Labs., Muskegon, MI, U.S.A.) and doubly-deionized, glass-distilled water were used for the LC analysis. Culture tubes (100 × 16 mm) with screw caps and tapered centrifuge tubes (15 ml) were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.) and were amberized with Amber-stain® No. 29-346 (Drakenfelt Colors Division of Ciba-Geigy, Washington, PA, U.S.A.) because furazolidone is light sensitive[10].

Heparinized swine blood was obtained from a local slaughterhouse at the time of slaughter. Plasma was spiked via syringe with the appropriate amount of a solution of furazolidone in water–methanol (70:30).

0021-9673/82/0000–0000/$02.75

Sample preparation

To an amber-colored culture tube were added 100 mg of sodium chloride (to prevent emulsion formation) and 2 ml of swine plasma. The plasma was extracted four times with 5 ml of ethyl acetate by shaking on a wrist-action shaker for 10 min. The ethyl acetate extracts were transferred with a Pasteur pipet to a 15-ml, amber-colored centrifuge tube, and the ethyl acetate was removed under a stream of nitrogen. LC mobile phase (200 μl) was added via a 250-μl syringe, and the tube was agitated for 1 min on a Vortex mixer to wash the surface with the solvent. The tube was centrifuged for about 3 min on a bench-top centrifuge to remove the small amount of insoluble material which was often present. The LC injector loop was overfilled with *ca.* 70 μl of the supernatant.

Chromatography

An Altex 100A pump (Altex, Berkeley, CA, U.S.A.), an Altex 210 injection valve fitted with a 50-μl loop, and a Whatman (Clifton, NJ, U.S.A.) Partisil PXS 10/25 ODS column were used. The pump was fitted with high-pressure inlet check valves (Altex) so that all plastic tubing could be replaced with 1/8-in. stainless steel. The mobile phase consisted of methanol–water (30:70) which was buffered to pH 4.0 with dibasic sodium phosphate (16.5 m*M*) and sodium citrate (13.1 m*M*). A flow-rate of 1.1 ml/min was used. The mobile phase was degassed by heating to 55°C under an atmosphere of helium. A Schoeffel SF770 Spectroflow variable-wavelength UV detector set at 362 nm and an electrochemical detector comprised of a TL5 glassy-carbon electrode and a LC4 amperometric controller (both from Bioanalytical Systems, West Lafayette, IN, U.S.A.) set at a potential of −0.75V relative to Ag/AgCl were employed.

RESULTS AND DISCUSSION

Since we could find no reports in the literature concerned with the distribution of furazolidone between plasma and red cells, we performed experiments to determine the distribution. In these experiments 10-ml samples of fresh, heparinized swine blood were spiked with the desired amount of furazolidone. The samples were immediately centrifuged, and 2 ml of the resulting plasma were analyzed. The amounts of furazolidone recovered were 96 and 193 ng/ml, respectively, when blood was spiked with 100 and 200 ng/ml. If one assumed that the amounts recovered were identical to those obtained with spiked plasma (see Table I and below), the actual amounts of furazolidone present in the plasma were 103 and 212 ng/ml, respectively. Since the volumes of plasma and red cells in swine plasma are about equal, these results suggest that the drug is distributed about equally between the plasma and the red cells.

Other experiments showed that the amount of furazolidone recovered from refrigerated whole blood decreased with time. Several 10-ml samples of heparinized blood were spiked with 100 ng/ml of the drug. Plasma from three samples was analyzed immediately and the remaining samples of whole blood were refrigerated. Plasma from these samples were analyzed in triplicate at various times. The average amounts of furazolidone were 92, 82, 74, and 44 ng/ml at 0, 24, 48, and 120 h, respectively. However, plasma containing furazolidone showed less than 10% de-

TABLE I

RECOVERY OF FURAZOLIDONE FROM PLASMA

Amount added (ng/ml)	*Recovery ± S.D. (ng/ml)*	*Recovery (%)*
200	182 ± 6.0	91.1
100	93.4 ± 4.1	93.4
60	55.6 ± 2.7	92.6
30	27.8 ± 1.1	92.4
10	9.47 ± 1.0	94.7

crease per day when refrigerated. The reason for this difference between whole blood and plasma is not known at this time, but is under study. The point is that whole blood containing furazolidone should be separated immediately and not stored prior to analysis. In this study plasma was spiked immediately before analysis.

A chromatogram from a plasma extract containing furazolidone and detected at 362 nm is shown in Fig. 1. Plasma from more than ten animals was examined; the chromatogram shown is typical. The response factor was $9.2 \cdot 10^{-5}$ a.u./ng, and the minimum detectable level (signal-to-noise ratio 2:1) was 0.5 ng which corresponded to 1 ng/ml of plasma. Detection was carried out at 362 nm because this is the wavelength of maximum absorption of furazolidone in the mobile phase and, more importantly, because of the absence of interfering peaks in the chromatograms. Detection at lower wavelengths, *e.g.* 254 nm, was unsatisfactory because of a large amount of background absorption. Similar observations have been made for nitrofurantoin in human plasma[11,12].

Recovery data for plasma spiked with furazolidone at levels which ranged from 10 to 200 ng/ml are shown in Table I. Each result shown represents the average of at least six determinations. The percent recoveries were similar for all levels examined. The standard deviation ranged from approximately 3 % at 200 ng/ml to 10 % at 10 ng/ml. For plasma which contained less than 100 ng/ml of furazolidone, identical recoveries were obtained with one less ethyl acetate extraction. Amounts of furazolidone were determined by comparing the sample peak with the average peak height from a standard solution which contained a similar amount of the drug. Alternatively, a standard curve could have been used since the detector response showed excellent linearity over the range examined.

In some experiments serum was substituted for plasma. Amounts of furazolidone added were 60, 100, and 200 ng/ml. Recoveries were identical, within experimental error, to those obtained with plasma.

In an effort to increase the sensitivity of the method, the use of a commercially available electrochemical detector was investigated[13]. It has been known for years that nitrofurans can be reduced electrochemically[14,15]. Polarography has, in fact, been used to determine furazolidone in feeds[16–18]. The polarographic behavior of a structurally similar nitrofuran, nitrofurantoin, has recently been investigated[19].

Because of the facile reduction of oxygen, efforts were made to exclude oxygen from the chromatographic system and from the sample when this detector was used. Oxygen dissolved in the mobile phase will increase the background current and thus

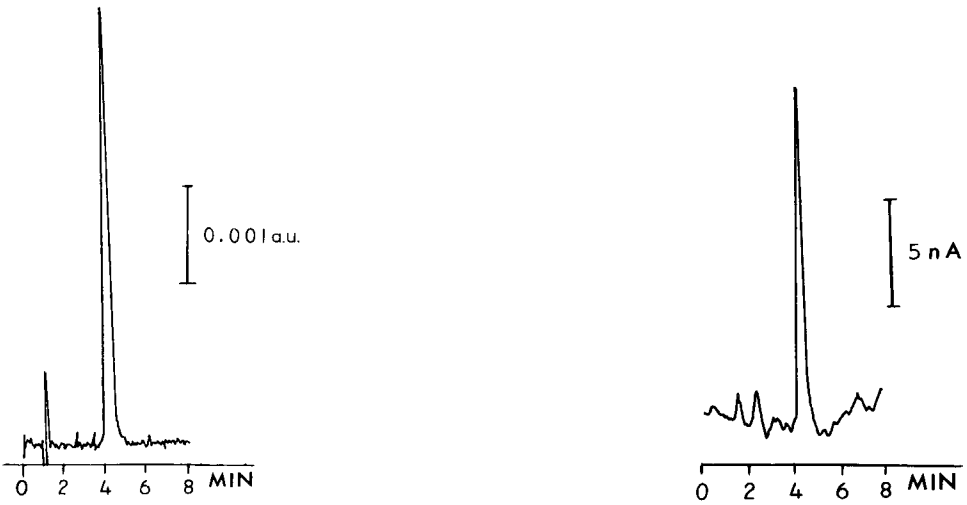

Fig. 1. Chromatogram obtained from plasma containing 100 μg/ml furazolidone and detected at 362 nm. Peak corresponds to 47 ng of furazolidone injected.

Fig. 2. Chromatogram obtained from injection of 50 ng of furazolidone dissolved in mobile phase and detected at a glassy-carbon electrode at a potential of −0.75 V *vs.* Ag/AgCl.

decrease sensitivity; oxygen dissolved in the sample will appear as a peak in the chromatogram. In addition to degassing the mobile phase, all plastic tubing was replaced with stainless steel to prevent permeation of air. Samples were degassed prior to injection by bubbling mobile phase-saturated nitrogen through the sample for 10 min.

We found that a glassy-carbon electrode at a potential of −0.75 V relative to Ag/AgCl gave the best signal-to-noise ratio for furazolidone with the chromatographic conditions used. Changing the pH of the mobile phase, the buffer-salt concentration, or the buffer composition, *e.g.* acetic acid–sodium acetate, did not increase the signal-to-noise ratio. Injection of standard solutions showed that the detector response was linear over the range examined (15–500 ng); the correlation coefficient from linear regression calculations was 0.999. As with UV detection at 362 nm, no peaks were present in the region where furazolidone eluted when plasma blanks were analyzed. The signal-to-noise ratio observed with this detector was similar to that found with 362 nm detection (Fig. 2). Similar detection limits for the two types of detectors used in this study is not surprising since Lund *et al.*[20] reported identical detection limits (3 ng) for a nitrobenzene derivative (nitrazepam) when detected at 254 nm and at a glassy-carbon electrode. Thus, electrochemical detection is a viable alternative to UV detection for furazolidone. Dual detection using both types of detectors would yield additional evidence to confirm the presence of furazolidone in plasma.

ACKNOWLEDGEMENTS

We thank Drs. W. Fiddler and O. W. Parks (USDA-SEA-AR) for helpful discussions. This work was supported by USDA-SEA-AR under Specific Coopera-

tive Agreement (No. 58-3244-0-129). We would also like to acknowledge the American Cancer Society Institutional Grant No. IN-88L for assistance in the purchase of the electrochemical detector.

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