Journal of Chromatography, 362 (1986) 141–145
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 655

Note

Determination of furazolidone in swine plasma, muscle, liver, kidney, fat and urine based on high-performance liquid chromatographic separation after solid-phase extraction on Extrelut® 1

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(Received March 14th, 1986)

Furazolidone [N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone], which is a nitrofuran, has been described as a potent antimicrobial agent for therapeutic and prophylactic purposes in pig, poultry and cattle[1]. Since furazolidone exhibits mutagenic and carcinogenic properties[2–4], the amount of furazolidone residue in edible products of food-producing animals treated with furazolidone should be minimal. This requires sensitive analytical methods for the detection of furazolidone in liver, muscle, fat and kidney. In addition, methods for the detection of furazolidone in plasma or urine may be needed for screening purposes and pharmacokinetic studies. Many different methods have been described for the detection of furazolidone in tissues, milk, eggs, plasma and urine[5–16].

Up till now, no suitable sensitive method has been described for the detection of furazolidone in plasma, muscle, liver, kidney, fat and urine. In this study, a relatively fast and sensitive high-performance liquid chromatographic (HPLC) method is described for the determination of furazolidone in plasma, muscle, liver, kidney, fat and urine of swine using a simple clean-up procedure based on solid-phase extraction on a commercially available disposable column. Only 1 g of sample is required.

EXPERIMENTAL

Chemicals and solvents

Water was purified over a Millipore Milli-Q water purification system. Ethyl acetate (LiChrosolv 868), acetonitrile (LiChrosolv 30), *n*-hexane (4391), acetic acid (62) and sodium acetate (6268) were purchased from Merck (Darmstadt, F.R.G.). Furazolidone was a gift from Orphahell (Mijdrecht, The Netherlands).

Acetate buffer solution (1.0 M). The solution contained 41 g of sodium acetate and 30 g of acetic acid in 1 l of water.

Stock solutions. Stock solutions of furazolidone in acetonitrile were prepared. The final concentration range was 1–1000 ng/ml eluent.

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Sample preparation

Plasma, urine. A 1-ml aliquot of plasma or urine was applied directly to a Merck Extrelut® 1 column (Merck 15371). After an equilibration period of 10 min, the drug was eluted with 6 ml of ethyl acetate. The eluate was collected in tubes and evaporated to dryness under nitrogen. The residue was dissolved in 300 μl of eluent.

Muscle, liver, kidney, fat. A 1-g quantity of frozen sample (liquid nitrogen) was pulverized and 2 × 6 ml of ethyl acetate were added and mixed for 1 min. After centrifugation for 5 min at 3000 *g*, the water phase was frozen (alcohol–dry ice). The ethyl acetate was collected in tubes and evaporated to dryness under nitrogen at room temperature. To remove fat from samples of liver, kidney and fat, the residue was dissolved in 5 ml of acetonitrile and 2 × 2 ml of hexane were added and mixed for 1 min. After centrifugation for 5 min at 3000 *g*, the hexane was removed and the acetonitrile was evaporated to dryness under nitrogen. The residues of samples of muscle, liver, kidney and fat were dissolved in 1 ml of a methanol–water mixture (25:75) and these solutions were applied to a Merck Extrelut 1 column. After an equilibration period of 10 min, the drug was eluted and prepared for HPLC as described for plasma and urine. Since furazolidone decomposes under the influence of daylight[17], brown glassware has been used and the experiments were performed under artificial yellow light (Pope FT 40W/16).

High-performance liquid chromatography

Liquid delivery system: Waters 6000A; automatic injection system: Waters 710B; detector: Kratos Spectroflow 773; absorbance recorded at 362 nm; recorder: Kipp BD 41.

Analytical column: Shandon Hypersil ODS 5 (250 × 4.6 mm I.D.) (Chrompack); precolumn: Brownlee Labs. OD-GU RP-18 (30 × 4.6 mm I.D.).

Eluent: water–acetate buffer–acetonitrile (675:75:250); flow-rate: 1 ml/min; run time: 15 min (urine: 20 min); injection volume: 50–100 μl.

RESULTS AND DISCUSSION

Because of the effective clean-up procedure, isocratic HPLC could be used. In blank samples of swine, no interfering peaks could be detected at 362 nm. Concentrations of furazolidone were calculated by comparing the peak heights from spiked samples with the peak heights from corresponding standard solutions, containing the same amounts of furazolidone. As shown in Fig. 1, linearity exists between the concentration of furazolidone and peak height in the range of 1–1000 ng/ml standard solution. Such a linearity was also found for spiked samples.

Recovery data for swine samples spiked with furazolidone in a concentration range of 1–1000 ng/ml (or ng/g) of sample are shown in Table I. Each result represents the average of six determinations. The mean overall recovery for plasma ($n=30$) is 96.4 ± 3.3, muscle ($n=30$): 98.8 ± 4.4, liver ($n=30$): 86.7 ± 6.4, kidney ($n=30$): 89.8 ± 4.9, fat ($n=30$): 81.8 ± 5.1 and urine ($n=24$): 91.2 ± 2.3.

The sensitivity of the method, expressed as three times the background of a blank sample, was 1 ng/ml for plasma, 2 ng/g for muscle, liver, kidney, fat and 25 ng/ml for urine.

In Fig. 2, the chromatograms of a blank and a spiked liver sample (5 ng/g) and a blank and spiked urine sample (25 ng/ml) are shown.

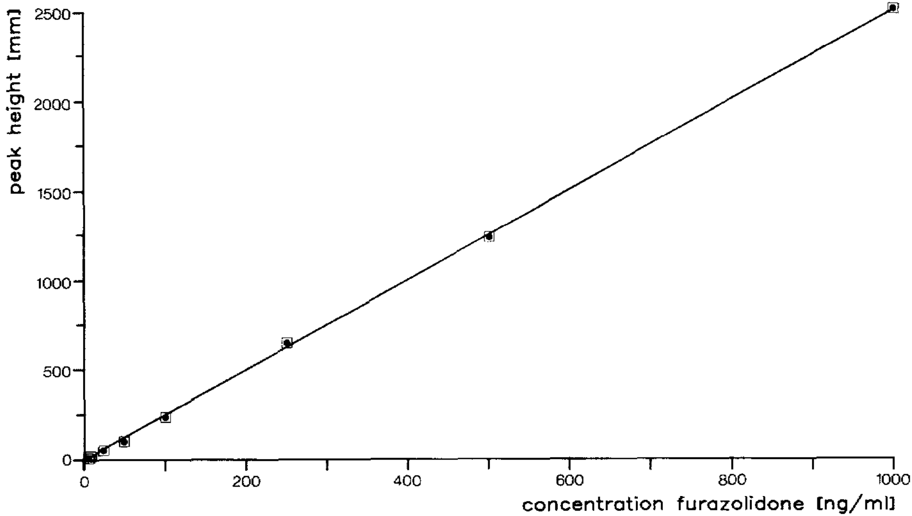

Fig. 1. Concentration of furazolidone in standard solution (ng/ml) as a function of peak height (mm) measured in the chromatogram.

The method proved its usefulness in a study in which a pig of approximately 90 kg body weight was treated with furazolidone by feed medication at a concentration of 300 mg/kg. Total daily feed intake was calculated on the basis of 4% of total body weight, dosing twice daily at 9.00 h and 16.00 h. Blood samples were collected via a syringe in the vena jugularis into heparinized tubes (Sarstedt 02.1065). Plasma was separated by centrifugation and immediately analysed, since a time-dependent decrease of furazolidone has been reported[16]. As an example, the chromatograms of furazolidone in plasma, 1 and 7 h after the last dosage at 9.00 h on day 10 of treatment, are shown in Fig. 3. Furazolidone concentrations were 104 and 6 ng/ml, respectively.

It can be concluded that the method described is suitable for the detection of furazolidone at the ng/ml or ng/g level in plasma, muscle, liver, kidney, fat and urine. The method is simple and requires only small amounts of sample and reagents. The

TABLE I

PERCENTAGE FURAZOLIDONE RECOVERED FROM SPIKED SAMPLES OF SWINE IN A FINAL RANGE OF 0–1000 ng/ml OR ng/g OF SAMPLE ($n=6$; MEAN ± S.D.)

Sample	*Concentration range of samples spiked with furazolidone*					
	1 ppm	*100 ppb*	*50 ppb*	*25 ppb*	*10 ppb*	*5 ppb*
Plasma	92.3 ± 2.3	95.3 ± 0.6	101.0 ± 2.4	–	96.2 ± 1.5	97.4 ± 1.2
Muscle	101.6 ± 5.3	98.9 ± 4.0	95.4 ± 3.6	–	99.4 ± 3.3	99.2 ± 4.4
Liver	91.2 ± 2.5	82.7 ± 2.7	88.4 ± 4.7	–	82.2 ± 5.1	89.2 ± 9.8
Kidney	83.5 ± 3.0	86.5 ± 1.2	91.8 ± 2.3	–	95.1 ± 1.8	91.9 ± 4.0
Fat	84.2 ± 4.4	84.0 ± 3.5	76.1 ± 2.7	–	78.6 ± 4.7	86.3 ± 1.2
Urine	88.8 ± 2.1	93.4 ± 0.6	90.8 ± 2.3	91.8 ± 0.9	–	–

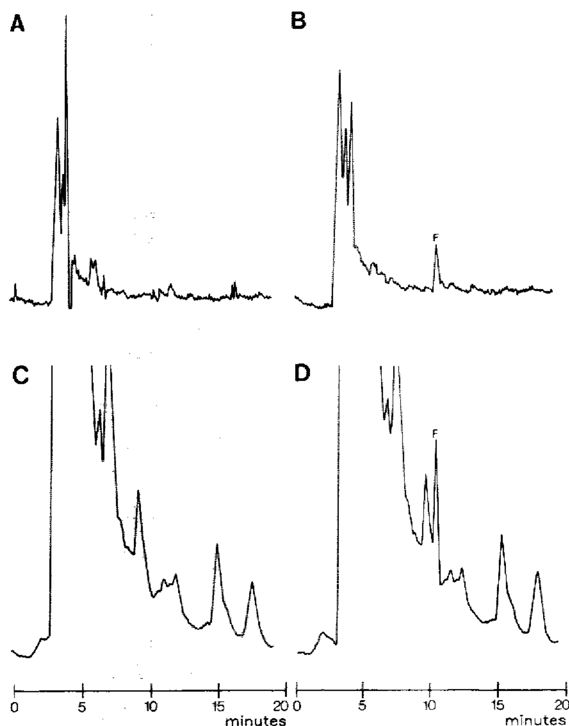

Fig. 2. High-performance liquid chromatograms of blank and spiked samples of swine liver (A: blank, B: 5 ng/g furazolidone) and swine urine (C: blank, D: 25 ng/ml furazolidone) (F = furazolidone). (A, B) λ = 362 nm; speed: 0.5 cm/min; sensitivity of detector: 0.002 a.u.f.s.; sensitivity of recorder: 10 mV; injection volume: 50 μl. (C, D) λ = 362 nm, speed: 0.5 cm/min; sensitivity of detector: 0.005 a.u.f.s.; sensitivity of recorder: 10 mV; injection volume: 50 μl.

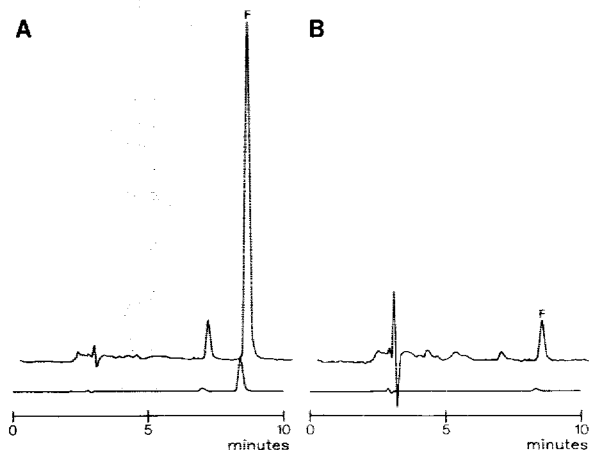

Fig. 3. High-performance liquid chromatograms of furazolidone in plasma of a pig, 1 h (A) and 7 h (B) after the last administration of furazolidone on day 10 of treatment (F = furazolidone). (A) λ = 362 nm; speed: 1 cm/min; sensitivity of detector, underline: 0.1 a.u.f.s., upperline: 0.01 a.u.f.s.; sensitivity of recorder: 10 mV; injection volume: 50 μl. (B) λ = 362 nm, speed: 1 cm/min; sensitivity of detector, underline: 0.1 a.u.f.s., upperline: 0.005 a.u.f.s.; sensitivity of recorder: 10 mV; injection volume: 50 μl.

total time that elapsed between sample collection and final determination of the amount of furazolidone varied between 30 and 60 min. The method may be used for screening purposes in plasma or urine of food-producing animals treated with furazolidone, and in pharmacokinetic studies.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. J. de Jong of the TNO-Institute of Animal Nutrition and Physiology (IGMB-TNO, Department ILOB), P.O. Box 15, 6700 AA Wageningen, The Netherlands for animal handling.

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