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Note

Determination of flumequine in biological fluids and meat by high-performance liquid chromatography

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Flumequine (6,7-dihydro-9-fluoro-5-methyl-1-oxo-1*H*,5*H*-benzochinoline-2-carboxylic acid) is an antimicrobial drug of the quinolone group, related to nalidixic and oxolinic acid [1,2] It is active against a broad spectrum of gram-negative bacteria [3] and is in use in veterinary therapy [4–7]

Until now there has been a lack of practical and suitable analytical methods to detect residues of flumequine in food Harrison et al [8] extracted acidified plasma with chloroform, followed by a second alkaline extraction of the organic layer Decolin et al [9] used a modified version of this method, which included a further clean-up step before extraction When Orlick and Frede [10] applied this method with UV detection to plasma endogenous sources of interference were observed at a flumequine level in plasma of 0 5 μg/ml. With the extraction method of Malisch [11] for liver a detection limit of 15 μg/g of liver was reached Beside UV detection, a spectrofluorimetric assay without chromatography was reported [8], which was linear over the concentration range 1–75 μg/ml for plasma and 1–50 μg/ml for urine

This paper describes a simple and rapid extraction method followed by chromatographic separation and fluorimetric detection

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EXPERIMENTAL

Reagents and materials

All chemicals and reagents were of analytical-reagent grade. Flumequine was supplied by Kettelhack Riker Pharma (Borken, F.R.G.). Urine, serum and meat were obtained from healthy pigs before and after treatment with flumequine.

Chromatographic conditions

The high-performance liquid chromatographic (HPLC) system consisted of a Spectra-Physics 8750 system (Santa Clara, CA, U.S.A.) connected to a fluorescence detector, a Spectra-Physics integrator and a plotter (Kompensograph III, Siemens, F.R.G.). The injection volume was 50 μl (Rheodyne, Cotati, CA, U.S.A., injection valve, Model 7125). The column (RP-8, 4 μm, 250 mm $\times$ 4 mm I.D.) and the pre-column (RP-18, 5 μm, 4 mm $\times$ 4 mm I.D.), both from Merck, (Darmstadt, F.R.G.), were kept at room temperature. A fluorescence detector (Shimadzu, RF-530, Kyoto, Japan) was operated at excitation and emission wavelengths of 320 and 380 nm, respectively.

The mobile phase was acetonitrile–phosphoric acid (25 mmol, 2.88 g/l) (70:30, v/v), adjusted with triethylamine to pH 3.5 (eluent A) and 100% acetonitrile (eluent B). Both eluents were filtered through a 0.5-μm filter (Millipore, Bedford, MA, U.S.A.) before use. They were used sequential at a flow-rate of 0.5 ml/min: eluent A, 1–10 min, eluent B, 10–30 min, eluent A, 30–40 min.

Extraction and clean-up

For body fluids (plasma and urine), 500 μl were acidified with 0.5 ml of 0.6 *M* hydrochloric acid and mixed well with 5 ml of chloroform for 30 s on a mechanical shaker. After separation of the two layers by centrifugation (1000 *g* for 5 min), the organic layer was evaporated at 60°C. The residue was dissolved in 250 μl of eluent A and filtered through a 5-μm filter before injection.

For meat and kidney, 10 g were homogenized in 40 ml of methanol–acetic acid (99.9:0.1, v/v) by Ultra-turrax. After centrifugation (3000 *g* for 10 min) the clear supernatant was mixed with 10 ml of hexane on a mechanical shaker for 15 s. The hexane layer was discarded and the organic layer was evaporated at 60°C and dissolved in 250 μl of eluent A.

RESULTS AND DISCUSSION

Several reports dealing with the determination of flumequine in different tissues and biological fluids have described the interference of other matrix compounds with flumequine. Matrix problems [10] were reduced by the combination of HPLC with a gradient eluent system and specific fluorescence de-

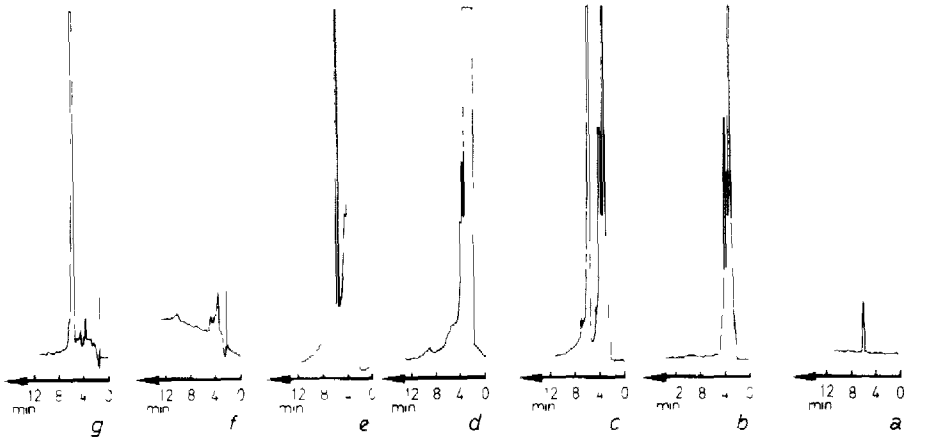

Fig 1 Representative chromatograms of (a) 0 02 μg flumequine per ml of 0 9% sodium chloride solution, (b) blank urine, (c) urine with 0 9 μg/ml flumequine, (d) blank serum, (e) serum with 1 5 μg/ml flumequine, (f) blank tissue (kidney) and (g) kidney with 1 3 μg/g flumequine The samples with flumequine were obtained from pigs 2 h after intravenous treatment with flumequine (12 mg/kg body weight)

tection No interferences from other serum, urine or tissue components were observed (Fig 1)

The detection limit of flumequine is ca 0 05 μg/ml in urine, 0 09 μg/ml in serum and 0 09 μg/g in kidney (at a signal-to-noise ratio of 3 1)

The linearity of determination ranges between the detection limit and 1 0 μg/ml, with a correlation coefficient greater than 0 992 The relative standard deviation of five repeated injections is below 8% in the corresponding concentration range The recovery from urine was consistently in the range 72–84%, and from and serum and kidney it was 32–41% This latter low recovery may be caused by protein binding as reported previously [7,10], but the results obtained demonstrate the suitability of the method for analytical monitoring

To exclude interferences, different extraction methods were chosen for biological fluids and kidney In contrast to serum samples (Fig 1d and e) only a few interferences could be seen in urine samples (Fig 1b and c) The extraction and clean-up method applied to urine and body fluids was not suitable for detecting flumequine residues in kidney because of interfering substances Hence methanol–acetic acid was chosen to extract flumequine from kidney followed by an additional clean-up step, which removed the fat-soluble substances Fig 1f and g show the chromatograms obtained, with only few peaks caused by matrix compounds

CONCLUSION

The advantage of the method presented here is the one-step extraction for body fluids, with a further clean-up required only for meat and kidney Inter-

fering signals were excluded, and the unsatisfactory detection limit with UV detection was improved by the specific fluorimetric detection Hence this extraction and detection procedure is suitable equally for pharmacokinetic studies and for meat inspection, as demonstrated by analysis of urine, serum and kidney samples from pigs after treatment with flumequine

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