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Simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by high-performance liquid chromatography

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ABSTRACT

A simple and rapid method for the simultaneous extraction and determination of sulfadiazine and trimethoprim in medicated fish feed by HPLC using sulfadimidine as internal standard is presented. The calibration curves were linear in the investigated areas, 1.25–10 mg/g of sulfadiazine and 0.25–2 mg/g trimethoprim. The recovery of sulfadiazine was 96–99%, and the recovery of trimethoprim 100–105%.

INTRODUCTION

Sulfadiazine and trimethoprim are often used in combination in the treatment of fish diseases, being administered by incorporation into the feed at a ratio of 5:1. This drug combination, together with the quinolones oxolinic acid and flumequine, and oxytetracycline, are the most commonly used drugs for this purpose in Norwegian fish farming [1]. In 1991, a total amount of 5679 kg of sulfadiazine–trimethoprim was used.

A number of procedures for the determination of sulfonamides in combination with trimethoprim in biological fluids and pharmaceutical preparations have been described [2–7].

HPLC methods for the determination of sulfadimidine (sulfamethazine) and sulfathiazole in feeds have also been published by Blanchflower and Rice [8], Conway [9], Houglum *et al.* [10], and Smallidge *et al.* [11]. Torel *et al.* [12] have analysed feed premixes containing sodium sulfamethazine, sodium sulfamethoxypyridazine and trimethoprim, but did not give any account of the procedure except for the HPLC conditions. McNally *et al.* [13] have published a method for determination of trimethoprim and sulfadiazine in medicated fish feed. The method is simple but time consuming.

The purpose of the present study was to develop a simple and rapid method for simultaneous determination of sulfadiazine and trimethoprim in fish feed.

MATERIALS AND METHODS

Materials and reagents

The starting point for samples was "clean" fish feed, *i.e.* feed containing no drugs. Sulfadiazine, trimethoprim, and the internal standard sulfadimidine were added to this unmedicated fish feed to prepare standard curves, and for recovery studies. The "real" samples to be analysed were taken from commercial medicated fish feed produced by Skretting (Stavanger, Norway).

All chemicals and solvents were of analytical

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or HPLC grade. Sulfadiazine and trimethoprim were supplied by Sigma. Sulfadimidine (sulfamethazine) (Serva) was used as internal standard.

A standard solution of sulfadiazine was made by dissolving 150 mg sulfadiazine in 50 ml 0.03 *M* sodium hydroxide–ethanol (1:1), and diluting to 150 ml with water. The trimethoprim standard solution was made by dissolving 50 mg trimethoprim in 10 ml 0.02 *M* H_3PO_4–CH_3CN (1:1), and diluting to 50 ml with water, and the standard solution of the internal standard sulfadimidine by dissolving 100 mg sulfadimidine in 5 ml acetone, and diluting to 100 ml with water. All standard solutions were put in an ultrasonic bath for 2 min before they were diluted with water.

Chromatographic conditions

The analyses were performed on a Perkin-Elmer HPLC system, consisting of a Series 400 solvent delivery system, an ISS 100 sampling system equipped with a Lauda RMT6 cooler (14°C) from Messgeräte Werk Lauda (Lauda-Königshafen, Germany), and an LC 235C UV detector (Perkin Elmer, Norwalk, CT, USA). The detector was operated at 270 nm. The integration was carried out using the software programme Omega-2 (Perkin-Elmer) in an Olivetti M 300 PC connected to a Star LC24-10 printer. The analytical column (stainless steel, 25 cm × 4.6 mm I.D.) and guard column (stainless steel, 2.0 cm × 4.6 mm I.D.) were packed with 5-μm particles of Supelcosil-LC-18-DB (Supelco, Bellafonte, PA, USA).

The mobile phase was 0.01 *M* aqueous Na_2HPO_4 pH 2.8–0.1% triethylamine in CH_3CN (79:21) at a flow-rate of 0.9 ml/min. Aliquots of 10 μl were injected onto the column for the determination of sulfadiazine and trimethoprim.

Sample preparation and clean-up

The feed sample, 1 g ground feed, was weighed into a 50-ml centrifuge tube with screw cap (NUNC). Internal standard sulfadimidine (1 ml of 1 mg/ml) and 3 ml 0.7% trichloroacetic acid (TCA) in acetone were added to the sample, which was then mixed well and left in an ultrasonic bath for 10 min at 40°C. The TCA solution was made by mixing 87 g TCA with 13 g water, to 0.7 ml of this solution was added 99.3 ml acetone.

The sample was transferred to a 500-ml volumetric flask. The centrifuge tube was washed and the volumetric flask filled to the mark with 0.01 *M* Na_2HPO_4, pH 3–CH_3CN (80:20). The pH in the Na_2HPO_4 solution was adjusted with 5 *M* H_3PO_4. The sample was well mixed, and an aliquot of 500 μl was filtered through a Costar spin-X centrifuge filter unit (low type) with 0.22-μm cellulose acetate binding by centrifugation for 1 min. Aliquots of 10 μl of the filtrate were injected onto the HPLC.

Calibration curves and recovery studies

The calibration curves for sulfadiazine and trimethoprim were made by spiking feed samples with standard solutions of sulfadiazine and trimethoprim to yield 1.25, 2.5, 5, 7.5 and 10 mg sulfadiazine per gram in feed, and 0.25, 0.5, 1, 1.5, and 2 mg trimethoprim per gram, respectively, in the samples. Duplicate samples were used. The recovery rates were determined by comparing peak height measurements of spiked feed to those of standard solutions. The linearity of the standard curves for sulfadiazine and trimethoprim in feed was tested using peak height ratios.

RESULTS AND DISCUSSION

Chromatograms of clean and spiked samples of fish feed are shown in Fig. 1 for sulfadiazine and trimethoprim. Fig. 2 shows a real (commercial) sample of medicated fish feed containing sulfadiazine and trimethoprim.

The linearity of the standard curves for sulfadiazine and trimethoprim in feed were tested using peak height ratios. The standard curves were linear in the investigated areas, 1.25–10 mg/g for sulfadiazine and 0.25–2 mg/g for trimethoprim. The correlation coefficients were $r = 0.9996$ for sulfadiazine in feed, and $r = 0.9994$ for trimethoprim.

Table I shows the recoveries and repeatabilities for sulfadiazine and trimethoprim from feed. The recovery of sulfadiazine from feed based on peak height varied from 96 to

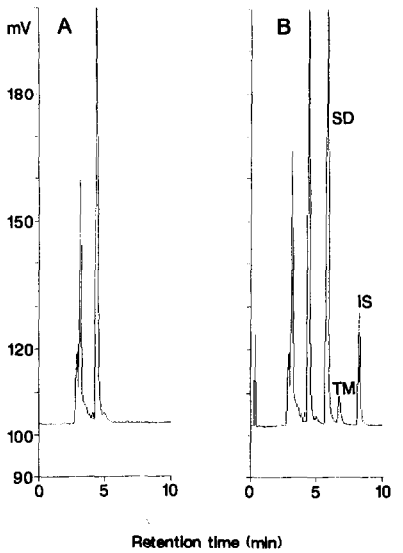

Fig. 1. Chromatograms of extracts from 1 g fish feed for the determination of sulfadiazine (SD) and trimethoprim (TM) with sulfadimidine as internal standard (IS). (A) Unspiked fish feed. (B) Fish feed spiked with 5 mg/g sulfadiazine and 1 mg/g trimethoprim.

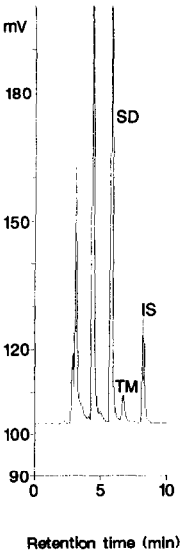

Fig. 2. Chromatogram of extract of 1 g "real" sample of fish feed containing sulfadiazine (SD) and trimethoprim (TM) with sulfadimidine as internal standard. The sample contains 5.0 mg/g sulfadiazine and 1.0 mg/g trimethoprim.

99%, the corresponding figures for trimethoprim being 100 and 105%. The standard deviation (S.D.) varied from 2.2 to 5.8%.

Table II shows the results of analysis of eight parallel samples of fish feed prepared so as to contain 5 mg/g sulfadiazine and 1 mg/g trimethoprim. The samples were found to actually contain 5.12 mg/g sulfadiazine and 1.04 mg/g trimethoprim on average, with an S.D. of 0.12 and 0.01, respectively.

McNally *et al.* [13] have previously published a method for the determination of trimethoprim and sulfadiazine in feed, in which the drugs are extracted with methanol by repeated extractions by slow rotation for 20 min, the procedure being repeated four times. The method is simple though time consuming. The present method is also simple, as well as being rapid and robust, and a large number of samples can easily be dealt with per day.

TABLE I

RECOVERY OF SULFADIAZINE (SDZ) AND TRIMETHOPRIM (TM) FROM FISH FEED

Material	No. of samples	Amount in spiked samples (mg/g)	Recovery (%)			
			SDZ		TM	
			Mean	S.D.	Mean	S.D.
Feed (1 g)	8	1.25	96	3.9		
	8	5.0	99	3.3		
	8	0.25			105	5.8
	8	1.0			100	2.2

TABLE II

ACTUAL CONTENT OF SULFADIAZINE (SDZ) AND TRIMETHOPRIM (TM) IN COMMERCIAL SAMPLES OF MEDICATED FISH FEED

The fish feed was prepared so as to contain 5 mg/g sulfadiazine and 1 mg/g trimethoprim.

Sample No.	Amount in samples (mg/g)	
	SDZ	TM
1	5.05	1.06
2	5.22	1.05
3	5.20	1.04
4	5.25	1.05
5	5.01	1.03
6	5.21	1.04
7	4.93	1.03
8	5.07	1.04
Average	5.12	1.04
S.D.	0.12	0.01

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