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Short communication

Simultaneous extraction and determination of florfenicol and the metabolite florfenicol amine in sediment by high-performance liquid chromatography

V. Hormazabal*, I. Steffenak, M. Yndestad

Division of Food Hygiene, Norwegian College of Veterinary Medicine, P.O. Box 8146 - Dep., N-0033 Oslo 1, Norway

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Abstract

A simple and rapid method for the simultaneous extraction and determination of florfenicol and the metabolite florfenicol amine in sediment by high-performance liquid chromatography is described. The calibration curves were linear, the recovery of florfenicol was 77-81% and the recovery of florfenicol amine was 82-86%. The limits of quantification for florfenicol and florfenicol amine in sediment were 1 μg/g and 0.5 μg/g, respectively.

Keywords: Florenicol; Florenicol amine

1. Introduction

Florfenicol is a fluorinated derivative of thiamphenicol. Experiments in which Atlantic salmon (*Salmo salar*) were given florfenicol have shown that the major metabolite is florfenicol amine [1]. Powers et al. [2] considered that florfenicol, because of its safety and high degree of efficacy, would become a drug of major significance in veterinary medicine, with special value in food producing animals.

The antibacterial activity of florfenicol against various fish pathogens has been determined by Fukui et al. [3], Yasunaga and Tsukahara [4], Yasunaga and Yasumoto [5], Kusuda et al. [6], and Inglis et al. [7]. The drug has been shown to be efficacious against a number of fish pathogens, and it is therefore of potential value in fish-farming.

Antibacterial agents are mostly administered to fish by incorporation into feed pellets. However, because diseased fish show reduced appetite or because of inadequate bioavailability of the agents, a relatively large portion of such drugs is released into the environment.

In order to monitor their presence, it is therefore of great importance to have methods to detect drugs and metabolites in fish-farm sediments.

A method for the determination of florfenicol and the metabolite florfenicol amine in fish tissues, muscle and liver has been described previously Hormazabal et al. [8].

The purpose of the present study was to develop an analytical method for the simultaneous extraction

*Corresponding author.

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and determination of the levels of florfenicol and the major metabolite florfenicol amine in sediment.

2. Materials and methods

2.1. Materials, reagents and chromatographic conditions

The samples used in this study consisted of marine sediment originating from an area with no fish farming activity and with no known effluent sources of antibiotics or chemotherapeutics.

All chemicals, solvents and the chromatographic conditions have been described previously [8].

3. Sample preparation and clean-up

The sediment sample (2 g) was weighed into a 50 ml centrifuge tube with screw cap (NUNC, Roskilde, Denmark). Internal standard thiamphenicol, 300μl of a standard solution of 100 μg/ml, was added. The sample was mixed and left standing overnight in the refrigerator (+4°C).

A 700-μl volume of water was added and the sample was shaken. A 3-ml volume of acetone was added and the sample was mixed for 5 s. The sample was then left with the extraction fluid for 15 min before being shaken vigorously for a further 5 s. The homogenate was then transferred to a 50-ml volumetric flask with 0.01 *M* Na_2HPO_4 (pH 3.0) - CH_3OH (80:20), and made up to the required volume. The sample was well mixed, and 500 μl was filtered through a $Costar_R$ spin-X centrifuge filter unit (low type) with 0.22 μm cellulose acetate binding by centrifugation for 2 min at 5600 *g* (Costar centrifuge). Aliquots of 30 μl of the filtrate were injected onto the HPLC.

Standard florfenicol and florfenicol amine were added to the samples for standard curves and recovery studies in addition to the internal standard, thiamphenicol. They were then left overnight. When water was added to the sample, the amount of water actually added depended on the amount of standard added to the sample. The volume of standard florfenicol and florfenicol amine added before the sample was left overnight, together with the volume of water added subsequently, totalled 700 μl.

To make standard curves and for recovery studies, the samples were made up to volume in 50 ml volumetric flasks, as described above. When carrying out analysis, one can avoid the need to transfer samples to volumetric flasks by making the samples up to volume in the 50 ml centrifuge tubes with screw cap (NUNC), the volume marked on the centrifuge tubes being sufficiently accurate for the analysis of a series of samples.

4. Calibration curves and recovery studies

The calibration curves for florfenicol and florfenicol amine were made by spiking sediment samples with standard solutions of florfenicol and florfenicol amine to yield 1, 2, 2.5, 5, 10, 20, 50 and 75 μg per gram in sediment samples. Duplicate samples were used. The recovery rates were determined by comparing results of analysis of the spiked sediment samples to those of standard solutions.

The linearity of the standard curves for florfenicol and florfenicol amine in sediment was tested using peak height measurements and the internal standard.

Table 1
Recovery of florfenicol (FF) and florfenicol amine (FFA) from spiked samples of sediment.

Sample	No. of sample	Amount in spiked samples (μg/g)	Recovery %			
			FF		FFA	
			Mean	S.D.	Mean	S.D.
Sediment	8	5	81	3.1		
(2g)	8	50	77	1.2		
	8	5			83	2.5
	8	50			86	2.1

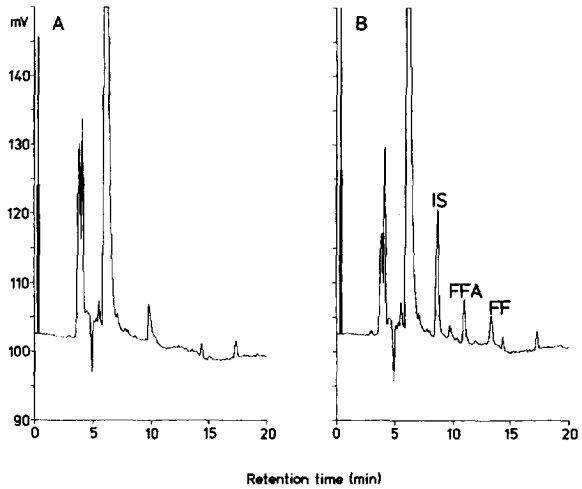

Fig. 1. Chromatograms of extract from 2 g sediment for the determination of florfenicol (FF) and florfenicol amine (FFA) with thiamphenicol as internal standard (I.S.). (A) Unspiked sediment. (B) Sediment spiked with 5 μg/g florfenicol and 5 μg/g florfenicol amine.

5. Results and discussion

Chromatograms of clean sediment sample and spiked samples are shown in Fig. 1.

As mentioned above, the linearity of the standard curves for florfenicol and florfenicol amine in sediment was tested using peak height measurements and the internal standard. The standard curves were linear in the investigated area, 1–75 μg/g of both florfenicol and florfenicol amine. The correlation coefficient for florfenicol in sediment was r=0.9997, the corresponding figure for florfenicol amine being r=0.9970.

Table 1 shows the recovery and the standard deviation for florfenicol and florfenicol amine in sediments. The recovery of florfenicol from sediment varied from 77 to 81%, and from 83 to 86% for florfenicol amine. The standard deviation varied from 1.2 to 3.1 and from 2.1 to 2.5 for florfenicol and florfenicol amine, respectively. The limits of quantitation (signal-to-noise ratio of 10) for florfenicol and florfenicol amine in sediment were 1 μg/g and 0.5 μg/g, respectively.

The samples of sediment spiked with the internal standard and the florfenicol and florfenicol amine standards were left overnight, because our experience has shown that these drugs bind slowly to the sediments. Exposure for several days increases the possibility of degradation of the drugs [9]. If the samples are extracted immediately, or shortly after the addition of standards, the recovery from the samples is almost 100%.

The method described is rapid, simple, selective and robust, and should be useful in investigating the effects of florfenicol and florfenicol amine on sediments. The potential number of samples that could be dealt with per day is limited only by the duration of the HPLC procedure. In addition, only small amounts of chemicals are required.

Acknowledgments

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