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Short communication

High-performance liquid chromatographic method for the rapid and simultaneous determination of sulfamonomethoxine, miloxacin and oxolinic acid in serum and muscle of cultured fish

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Abstract

A rapid method for the simultaneous determination of sulfamonomethoxine (SMM), miloxacin (MLX) and oxolinic acid (OA) in serum and muscle of cultured fish by high-performance liquid chromatography has been developed. A Hisep shielded hydrophobic phase column (15 cm×4.6 mm I.D.) and a mobile phase of 0.05 *M* citric acid–0.2 *M* disodium hydrogenphosphate buffer, pH 2.5 in 10 m*M* tetra-*n*-butyl ammonium bromide–acetonitrile (85:15) with ultraviolet detection at 265 nm were used. The recoveries of SMM, MLX and OA from serum and muscle samples were 72–101%. The detection limits of the three drugs were 0.05–0.1 μg/ml or g of sample.

Keywords: Sulfamonomethoxine; Miloxacin; Oxolinic acid

1. Introduction

In recent years, various kinds of fish drugs have been used for the control of fish diseases in fish culture in Japan. As a result, the drugs remaining in the tissues of fish have raised questions about not only the quality but also the safety of cultured fish as food. According to the Law of the Slaughterhouse and Food Hygiene for domestic animals in our country, their safety as food has been checked by a microbiological assay [1,2] for the detection of residual antibacterial drugs. However, very little attention has been paid to the residual drugs in cultured fish when compared with those in domestic animals. In addition to the residual drug assays, the efficiency with which fish drugs are used has been evaluated by pharmacokinetic analyses of drug levels in serum [3–9] in order to obtain information about the appropriate administration of the drugs. Therefore, in clinical applications for fish farms, the drug concentrations should be closely monitored for the optimal therapeutic effect.

Sulfamonomethoxine (SMM), miloxacin (MLX), and oxolinic acid (OA) are typical synthetic antibacterial agents widely used in fish culture. Although many papers have been published concerning the assay of these drugs, there are few methods for their simultaneous determination. Moreover, these methods are not adequate for the rapid detection of drug residues or for monitoring drug concentrations: microbiological assays [1,2,10–14] and fluorometry [15–17] lack sensitivity and specificity, and high-

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performance liquid chromatography (HPLC), although sensitive, requires long pretreatments [7,9,18–30]. Therefore, a rapid and sensitive method for the determination of these drugs in blood serum and muscle is still needed.

The present paper describes a rapid, sensitive and simultaneous analysis for SMM, MLX and OA in serum and muscle of cultured fish by HPLC. The method uses samples that require simple pretreatment for muscle and no pretreatment for serum.

2. Experimental

2.1. Chemicals

MLX, SMM and OA were generously supplied by Sumitomo Seiyaku (Osaka, Japan), Daiichi Seiyaku (Tokyo, Japan) and Tanabe Seiyaku (Osaka, Japan), respectively. All reagents were of analytical grade (Wako, Osaka, Japan).

2.2. Apparatus and conditions

The HPLC system was Waters 625 LC system (Waters, Milford, MA, USA). The analytical column was a Hisep shielded hydrophobic phase column, 15 cm×4.6 mm I.D., 5 μm particle size (Supelco, Bellefonte, PA, USA), protected with a guard column, 2 cm×4.6 mm I.D., packed with the same material. Peak areas were quantified by means of a Waters 741 data module.

The flow-rate was 1.0 ml/min, and the UV detector was set at 265 nm and 0.01 a.u.f.s. The sample volume injected onto the column was 20 μl. The analysis was performed at ambient temperature.

Standard solutions were 1 mg/ml SMM in methanol, 1 mg/ml MLX in 1% sodium carbonate, and 1 mg/ml OA in 0.1 *M* borate buffer, pH 10.0. The solutions were kept at −20°C. Each solution was diluted to the required concentration with drug-free serum and muscle samples before use.

2.3. Analytical procedure

Serum spiked with the drugs and blank serum samples were filtered through 0.45-μm disposable syringe filter units equipped with a cellulose acetate membrane (Advantec, Tokyo, Japan). A 50-μl portion of the filtrate was injected directly onto the chromatograph under the conditions described above. One gram of muscle spiked with the drugs and blank muscle samples were put into 10-ml centrifuge tubes, 1 ml of acetonitrile–tetrahydrofuran (95:5) was added, and the samples were homogenized with a Pencil Mixer (Iuchi, Osaka, Japan) for 2 min. After centrifugation at 4000 rpm (1500 *g*) for 5 min, the supernatants were filtered through the syringe filter units. A 20-μl portion of the filtrate was injected onto the chromatographic system.

3. Results and discussion

The separation of SMM, MLX and OA was carried out by reverse-phase ion-pair chromatography with the Hisep column, consisting of a polymeric hydrophilic/hydrophobic phase bound on silica gel. Good separation of SMM, MLX and OA with relatively short retention times was achieved at room temperature with a mobile phase consisting of 0.05 *M* citric acid–0.2 *M* disodium hydrogenphosphate buffer, pH 2.5 in 10 m*M* tetra-*n*-butyl ammonium bromide–acetonitrile (85:15). Fig. 1 shows

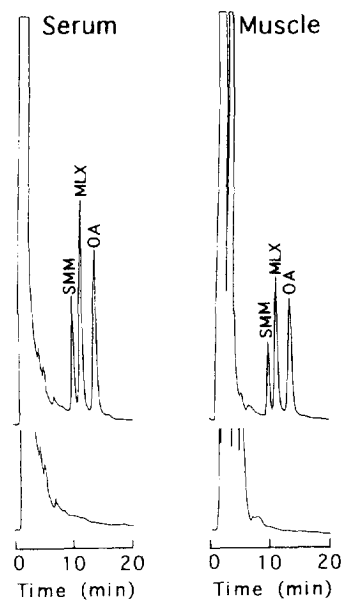

Fig. 1. Typical chromatograms of eel serum and muscle, both unspiked and spiked with sulfamonomethoxine (SMM), miloxacin (MLX) and oxolinic acid (OA) (2.0 μg/ml or g) and their blank samples.

Table 1
Recovery of sulfamonomethoxine, miloxacin and oxolinic acid from spiked serum and muscle of cultured fish

Drug	Recovery (%)	
	Serum	Muscle
Sulfamonomethoxine	101 (7.0)	72.7 (3.4)
Miloxacin	100 (6.9)	71.6 (6.8)
Oxolinic acid	95.1 (3.5)	79.5 (6.0)

Values in parentheses are coefficients of variation (%).

typical chromatograms of eel serum and muscle, both unspiked and spiked with SMM, MLX and OA (2.0 μg/ml or g). The proteins eluted with the void volume, and no interfering peaks were observed in the blank chromatograms. The retention times were 9.5 min for SMM, 11.5 min for MLX and 14 min for OA.

Correlation coefficients for the serum standard calibration curves of SMM, MLX and OA were 0.999 or higher; thus, the linearities were good. Standard calibration curves for the three drugs in the serum sample tested were linear at least over the range 0.25 to 50 μg/ml. The same results were obtained for the muscle sample.

Satisfactory recoveries of SMM, MLX and OA were obtained from all samples tested (Table 1). For all three drugs, the limits of detection (signal-to-noise ratio of 3) were 0.05 μg/ml for serum samples and 0.1 μg/g for muscle samples.

This HPLC method did not require time-consuming and complex extraction procedures and, moreover, did not cause column clogging, peak broadening or variation of retention time throughout the analysis.

In conclusion this method is suitable not only for rapid detection of the drug residues in muscle, the main edible part, but also for monitoring of drug levels in the serum of cultured fish for pharmacokinetic studies.

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