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Short communication

Simultaneous determination of sulphamonomethoxine and its N^4-acetyl metabolite in blood serum by high-performance liquid chromatography with direct injection

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Abstract

A high-performance liquid chromatographic method with direct injection has been developed for the simultaneous determination of sulphamonomethoxine and its N^4-acetyl metabolite in serum of animals and fish. A HISEP shielded hydrophobic-phase column (15 cm × 4.6 mm I.D.), a mobile phase of 0.05 *M* citric acid–0.2 *M* disodium hydrogenphosphate–acetonitrile (70:15:15, v/v), and ultraviolet detection at 265 nm were used. The standard calibration curves in serum of chicken, pig, cattle, rainbow trout and yellowtail were linear over the range 0.5–20 μg/ml. The recoveries of sulphamonomethoxine and its N^4-acetyl metabolite from all serum samples determined at different concentrations (0.5, 2.0 and 10.0 μg/ml) were 93–103% and 90–103%, respectively. The lowest measurable sulphamonomethoxine and N^4-acetyl metabolite concentrations were 0.04 and 0.1 μg/ml, respectively, for all serum samples.

1. Introduction

Sulphamonomethoxine (SMM) is a sulphonamide derivative used for domestic animals and cultured fish as therapeutic and prophylactic agent in Japan. Recently, the efficiency of the use of sulphonamide derivatives in animals, including fish, has been evaluated by pharmacokinetic analysis of serums level [1–6]. Especially in clinical applications, the drug concentration should be closely monitored for the optimal therapeutic effect.

Several papers have been published on the sensitive assay of SMM by high-performance liquid chromatography (HPLC) [7–13]. However, these methods are not adequate for monitoring drug concentrations: the HPLC methods require long pretreatment procedures, such as protein precipitation, liquid- and solid-phase extractions. Additionally, there are few reports on the simultaneous determination of SMM and its N^4-acetyl metabolite. The N^4-acetyl metabolite, N^4-acetylsulphamonomethoxine (AcSMM), has no antibacterial activity and is less water-soluble than the parent drug, potentially leading to crystalluria which may cause renal damage.

This paper describes the development of a more rapid HPLC assay, involving direct injection, for SMM and its N^4-acetyl metabolite in

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the serum of animals and fish, without sample pretreatment.

2. Experimental

2.1. Chemicals

Sulphamonomethoxine (SMM) was obtained from Daiichi Pharmaceutical (Tokyo, Japan). N^4-Acetylsulphamonomethoxine (AcSMM) was synthesized according to the method described in Japanese Pharmacopoeia (1981) [14]. Other chemicals were of analytical grade or HPLC grade.

2.2. Apparatus

The HPLC system consisted of a Jasco PU-980 pump and UV-970 variable-wavelength absorbance detector (Japan Spectroscopic, Tokyo, Japan). The samples were injected with a Rheodyne 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, 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 with a Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan). The system was operated at room temperature.

2.3. Operating conditions

The mobile phase consisted of 0.05 *M* citric acid–0.2 *M* disodium hydrogenphosphate–acetonitrile (70:15:15, v/v). The pH was not adjusted. The flow-rate was 1.0 ml/min, and the UV detector was set at 265 nm and 0.02 AUFS. The sample volume injected onto the column was 20 μl.

2.4. Standard solutions

The standard solutions of SMM and AcSMM were prepared at a concentration of 1000 μg/ml in methanol. The solutions were diluted to the required concentrations with distilled water before use.

2.5. Assay procedure

Serum spiked with the drug and blank serum samples were filtrated through 0.45-μm disposable syringe filter units equipped with cellulose acetate membranes (Advantec, Tokyo, Japan). A 20-μl portion of the filtrate was directly injected onto the chromatographic system under the conditions described above.

2.6. Calibration and recovery

Standard calibration curves for SMM and AcSMM in the range 0.5–20 μg/ml were prepared with drug-free serum from chicken, pig, cattle, rainbow trout and yellowtail. The recoveries of SMM and AcSMM were determined from serum samples spiked at 0.5, 2.0 and 10.0 μg/ml.

3. Results and discussion

Gisch et al. [15,16] and Ueno et al. [17] reported that the HISEP column was useful for direct-injection HPLC analyses of drugs in

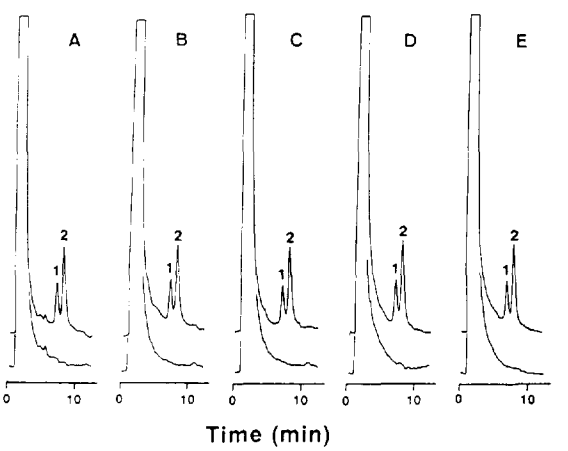

Fig. 1. Typical chromatograms of (A) chicken, (B) pig, (C) cattle, (D) rainbow trout and (E) yellowtail serum samples spiked with 2.0 μg/ml sulphamonomethoxine (2), 2.0 μg/ml N^4-acetylsulphamonomethoxine (1) and their blank serums.

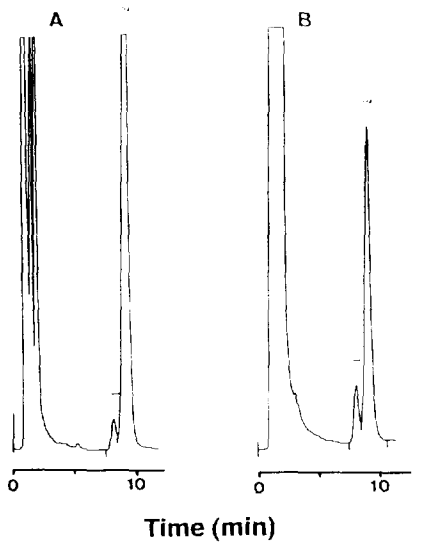

Fig. 2. Chromatograms of real samples. (A) chicken serum fed a diet containing 0.1% of sulphamonomethoxine; (B) rainbow trout serum taken 12 h after oral administration of sulphamonomethoxine (300 mg/kg). 1 = N^4-acetylsulphamonomethoxine; 2 = sulphamonomethoxine.

human, animals and fish serum. In our experiments, we also used the HISEP column as analytical column. Then we attempted to find suitable separating conditions such as the ratio of the constituents, the concentration and the pH of the mobile phases and the column temperature. The optimum combination of separation and retention time for SMM and AcSMM was achieved by using 0.05 *M* citric acid–0.2 *M* disodium hydrogenphosphate–acetonitrile (70:15:15, v/v). Satisfactory separation was obtained at room temperature with this mobile phase.

Fig. 1 shows typical chromatograms of SMM and AcSMM in serum of chicken, pig, cattle, rainbow trout and yellowtail (2.0 μg/ml), and their blanks. The retention times of SMM and AcSMM were 7.2 and 8.6 min, respectively. Proteins were eluted unretained. No interfering peaks were observed in the chromatograms of blank serums. Chromatograms of real samples, i.e. serum samples from chicken and rainbow trout treated with SMM, are shown in Fig. 2.

Table 1
Recoveries of sulphamonomethoxine and its N^4-acetyl metabolite in serum samples from chicken, pig, cattle, rainbow trout and yellowtail

Animal	Recovery (%)					
	0.5		2.0		10.0	
	SMM	AcSMM	SMM	AcSMM	SMM	AcSMM
Chicken	97.5 (2.9)	95.5 (2.9)	99.2 (4.1)	98.2 (4.5)	97.2 (2.2)	94.5 (2.2)
Pig	103 (1.7)	92.9 (4.8)	97.2 (3.2)	94.0 (3.4)	97.3 (4.2)	95.1 (3.9)
Cattle	101 (4.0)	100 (3.7)	99.5 (6.3)	103 (6.9)	98.6 (4.4)	99.0 (2.1)
Rainbow trout	93.7 (3.8)	90.8 (4.6)	98.3 (2.1)	93.0 (2.5)	97.7 (5.3)	98.0 (5.0)
Yellowtail	103 (2.5)	102 (3.8)	97.8 (5.1)	93.0 (2.5)	93.2 (1.3)	90.1 (1.3)

Values in parentheses are coefficients of variation (%).

The correlation coefficients of the serum standard calibration curves of SMM and AcSMM for chicken, pig, cattle, rainbow trout and yellowtail were 0.9997, 0.9999, 0.9997, 0.9996 and 0.9999, and 0.9999, 0.9999, 0.9994, 0.9996 and 0.9999, respectively; thus, good linearity was found. Standard calibration curves for SMM and AcSMM in all serum samples tested were linear at least over the range of 0.5–20 μg/ml.

The recoveries of SMM and AcSMM determined at different concentrations (0.5, 2.0 and 10.0 μg/ml) are listed in Table 1. Satisfactory recoveries of SMM and AcSMM were obtained from all serum samples.

The intra- and inter-day coefficients of variation (C.V.) were evaluated by replicate ($n = 5$) analyses of samples at 2.0 μg/ml. The intra-day C.V. of SMM and AcSMM varied between 3.2 and 6.6%. The inter-day C.V.s, evaluated over six days, were 4.8, 4.8, 7.0, 7.1 and 6.4% for SMM and 5.2, 5.8, 7.0, 5.1 and 6.2% for AcSMM from chicken, pig, cattle, rainbow trout and yellowtail serums, respectively. The lowest measurable SMM and AcSMM concentrations were 0.04 and 0.1 μg/ml, respectively, for all samples (signal-to-noise ratio of 3).

Even after more than 800 sample injections, this HPLC method did not show column-clogging, peak broadening or variation of retention times.

In conclusion, this method is suitable for the monitoring of SMM and its N^4-acetyl metabolite in animals and fish, and also for pharmacokinetic studies.

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