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Short Communication

Determination of oxytetracycline in blood serum by high-performance liquid chromatography with direct injection

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ABSTRACT

A direct injection analysis by high-performance liquid chromatography has been developed for oxytetracycline in serum of animals and fish. A Hisep shielded hydrophobic phase column (15 cm × 4.6 mm I.D.) and a mobile phase of methanol–0.2 *M* oxalic acid (10:90, v/v, pH 7.0) with ultraviolet detection at 360 nm were used. The standard calibration curves in serum of chicken, hog, cattle and rainbow trout were linear over the range 0.1–20 μg/ml. The recoveries of oxytetracycline from all serum samples determined at two different concentrations (0.5 and 2.0 μg/ml) were 88–103%. The detection limit was 0.05 μg/ml for every serum sample.

INTRODUCTION

Oxytetracycline is an antibacterial drug used in domestic animals and cultured fish as a therapeutic and prophylactic agent in Japan. Recently, the efficiency of the use of oxytetracycline in animals, including fish, has been evaluated by pharmacokinetic analysis of serums level [1–7]. Especially in clinical applications, the drug concentration should be closely monitored for the optimal therapeutic effect.

Many papers have been published concerning the assay of oxytetracycline. However, these methods are not adequate for the monitoring of drug concentrations: bioassay [1–5] and fluorometry [8], which are commonly used, lack sensitivity and specificity, and high-performance liquid chromatography (HPLC) [6,7,9–15] is sensitive, but requires long pretreatment. Therefore a rapid and sensitive method for the determination of oxytetracycline in blood serum is still needed.

This paper describes the development of a more rapid HPLC assay, involving direct injection, for oxytetracycline in the serum of animals and fish, without pretreatment.

EXPERIMENTAL

Chemicals

Oxytetracycline hydrochloride was purchased

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from Sigma (St. Louis, MO, USA). Other chemicals were of analytical grade or of HPLC grade.

Apparatus

The HPLC system consisted of a Jasco 880-PU pump (Japan Spectroscopic, Tokyo, Japan), a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA) with a 100-μl loop, and a Gilson 311A variable-wavelength UV detector (Gilson, Villiers-le-Bel, France). 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 Chromatopac C-R3A integrator (Shimadzu, Kyoto, Japan).

Operating conditions

The mobile phase was methanol–0.2 *M* oxalic acid (10:90, v/v). The pH of the mobile phase was adjusted to 7.0 with 28% aqueous ammonia. The flow-rate was 1.0 ml/min, and the UV detector was set at 360 nm and 0.02 a.u.f.s. The sample volume injected on the column was 100 μl.

Standard solution

The standard solution of oxytetracycline was prepared at a concentration of 100 μg/ml in distilled water and kept at −20°C. The solution was diluted to required concentrations with distilled water before use.

Assay procedure

Serum spiked with the drug and blank serum samples were filtered through 0.45-μm disposable syringe filter units equipped with cellulose acetate membrane (Advantec, Tokyo, Japan). A 100-μl portion of the filtrate was directly injected into the chromatograph under the conditions described above.

Calibration and recovery

Standard calibration curves for oxytetracycline in the range 0.1–20 μg/ml were prepared with drug-free serum from chicken, hog, cattle and rainbow trout. The recoveries of oxytetracycline were determined from serum samples spiked at 0.5 and 2 μg/ml.

RESULTS AND DISCUSSION

Gisch and co-workers [16,17] reported direct sample injection HPLC analyses for carbamazepine, phenobarbital, theophylline and trimethoprim in human and fetal bovine serum by using a Hisep shielded hydrophobic phase column, consisting of a polymeric hydrophilic/hydrophobic phase bonded on silica gel. In our preliminary experiments we applied the above method for the assay of oxytetracycline in serum. However, a suitable separation could not be obtained with the reported mobile phases. We thus varied the conditions, such as the ratio of constituents, the concentration and pH of the mobile phase and the column temperature. The best separation and retention time of oxytetracycline were achieved by the use of methanol–0.2 *M* oxalic acid (10:90, v/v, pH 7.0). Satisfactory separation was obtained at room temperature with this mobile phase, and the absorption maximum of oxytetracycline was at 360 nm.

Fig. 1 shows typical chromatograms of oxytetracycline in the serum of chicken, hog, cattle and rainbow trout (2.0 μg/ml), and their blank se-

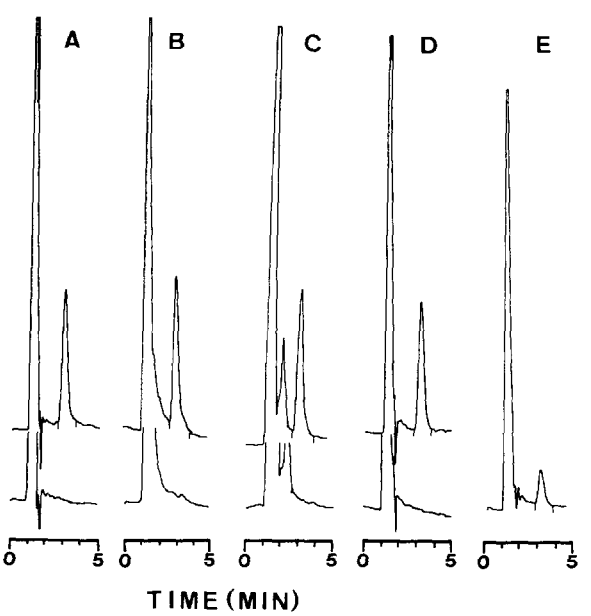

Fig. 1. Typical chromatograms of (A) chicken, (B) hog, (C) cattle and (D) rainbow trout serum samples spiked with 2.0 μg/ml oxytetracycline and their blank serums. (E) Chromatogram obtained from chicken serum collected 24 h after the feeding of oxytetracycline (500 ppm).

TABLE I

RECOVERIES OF OXYTETRACYCLINE IN SERUM SAMPLES FROM CHICKEN, HOG, CATTLE AND RAINBOW TROUT

Values in parentheses are coefficients of variation (%).

Added (μg/ml)	*n*	Recovery (%)			
		Chicken	Hog	Cattle	Rainbow trout
0.5	5	87.6 (4.8)	93.1 (1.2)	103 (3.9)	92.7 (0.7)
2.0	5	95.4 (2.4)	94.5 (3.2)	94.5 (1.7)	96.8 (5.8)

rums. The retention time of oxytetracycline was 3.80 min. The proteins were eluted unretained. No interfering peaks were observed in the blank chromatograms.

The correlation coefficients for the serum standard calibration curves of chicken, hog, cattle and rainbow trout were 0.9992, 0.9999, 0.9999 and 0.9998, respectively; thus the linearities were good. Standard calibration curves for oxytetracycline in all serum samples tested were linear at least over the range 0.1–20 μg/ml.

The recoveries of oxytetracycline determined at two different concentrations (0.5 and 2.0 μg/ml) are listed in Table I. Satisfactory recoveries of oxytetracycline were obtained from all serum samples tested. The intra- and inter-day coefficients of variation (C.V.) were evaluated by replicate ($n = 5$) analyses of samples at 2 μg/ml. The intra-day C.V. of every sample was less than 3%. The inter-day C.V. evaluated over six days, were 2.7, 2.3, 1.7 and 4.2% for chicken hog, cattle and rainbow trout, respectively. The detection limit (signal-to-noise ratio of 3) of the method was 0.05 μg/ml for every serum sample.

This HPLC method did not require time-consuming and complex extraction and, moreover, did not cause column clogging, peak broadening or variation of retention times throughout the analyses (over 1000 sample injections).

In conclusion this method is suitable for the monitoring of oxytetracycline in animals and fish, and also for pharmacokinetic studies.

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