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Short communication

Rapid liquid chromatographic determination of oxytetracycline in milk

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Abstract

A simple method for the determination of residual oxytetracycline (OTC) in milk by high-performance liquid chromatography (HPLC) was developed. The sample preparation could be made without complex extraction and clean-up procedures. A LiChrospher 100 RP-8 end-capped column and a mobile phase of acetonitrile-acetic acid-water (28:4:68, v/v/v) with a photo-diode array detector was used. The average recoveries from spiked OTC (0.1, 0.5 and 1.0 μg/ml) were in excess of 89.8% with coefficients of variation between 0.6 and 4.1%. The limit of detection was 0.05 μg/ml. The total time required for the analysis of one sample was below 10 min. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Oxytetracycline; Antibiotics

1. Introduction

Oxytetracycline (OTC) is applied to the prevention or the treatment of mastitis and metritis in cows [1]. As a result, there is concern that residues of the compound may be retained in the milk. To prevent any health problems with consumers, FAO/World Health Organization (WHO), US Food and Drug Administration (FDA), European Union (EU) and Japan (Japanese Food Sanitation Laws) have been established its maximum residue limit (MRL) in milk (0.1 μg/ml) [2–5]. The analytical method for OTC residue monitoring programs should be accurate, simple, economical in time and cost, and capable of detecting the residues below MRLs.

For quantification of OTC residues, high-performance liquid chromatography (HPLC) methods are effective in the monitoring many veterinary drugs and several HPLC methods have been reported for the determination of OTC in various foods [6–15]. However, the extraction and clean-up of these methods involves numerous and varying analytical steps which are time consuming and do not permit monitoring of a large number of samples. A better method for determination of OTC in milk is lacking so far. Because it is highly nutritious, cheap and readily available, milk is a very important food. Monitoring of milk for OTC residues must be rigorous and economical, hence the need for better analytical techniques.

The present paper describes a simple procedure for HPLC determination of residual OTC in milk without complex extraction and clean-up techniques. The

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detection is performed by using a photo-diode array detector which is able to detect a wide range of molecules and ensure their identification [16–18].

2. Experimental

2.1. Materials and reagents

Bottled/paper-packed milk served as a sample, and was stored in a refrigerator until analysis. OTC standard was obtained from Sigma (St. Louis, MO, USA). Other chemicals were obtained from Wako (Osaka, Japan): distilled water and acetonitrile were of HPLC grade. Trichloroacetic acid (TCA, 100%, w/v) solution and acetic acid were of analytical chemical grade. As a extraction/deproteinization-solution, 20% (v/v) TCA solution (diluted with distilled water) was used.

A stock standard solution of OTC was prepared by accurately weighing 10 mg, dissolving it in 100 ml of 1% (v/v) acetic acid (in water) solution. Working standard solutions were prepared by diluting the stock solution with distilled water. These solutions stored in a refrigerator and were stable for up to one month.

2.2. Apparatus

A disposable syringe filter unit (DISMIC-25cs, 0.45 μm hydrophilic cellulose acetate membrane) was obtained from Advantec Toyo (Tokyo, Japan).

Analyses of standard and extracted OTC were conducted using a Jasco HPLC system (Model PU-980 pump and DG-980-50 degasser) (Jasco, Tokyo, Japan) equipped with an SPD-M10A $_{VP}$ diode array detector (Shimadzu, Kyoto, Japan) interfaced with an Fujitsu FMV-5133D7 personal computer (Fujitsu, Tokyo, Japan). The separation was performed on a LiChrospher 100 RP-8 end-capped (e) (5 μm) column (250×4 mm I.D.) (Merck, Darmstadt, Germany) with a guard column (4×4 mm I.D.) (Merck) using acetonitrile–acetic acid–water (28:4:68, v/v/v) as the mobile phase at a flow-rate of 1.0 ml/min at ambient temperature.

2.3. Procedure

A 1-ml sample was placed into a 5-ml test tube and shaken intensively with 1.5 ml of 20% (v/v) TCA solution for 30 s. The mixture was filtered through a 0.45-μm disposable syringe filter unit. A 20-μl volume of the filtrate was directly injected into the HPLC system.

2.4. Recovery test

The recoveries of OTC were determined from blank milk samples spiked at 0.1, 0.5 and 1.0 μg/ml. These spike levels were prepared by adding 100 μl of three standard solutions of OTC (OTC 1.0, 5.0 and 10.0 μg/ml), respectively, to separate 1.0-ml portions of the sample. These fortified samples were allowed to stand at 4°C for 12 h after OTC addition followed by mixing. In this test, blank milk samples used for ''inter-day'' and ''intra-day'' were five different batches and one of the batches, respectively.

3. Results and discussion

3.1. HPLC operating conditions

A problem of silica-based reversed-phase HPLC is that OTC is easy to detect as the tailing peak owing mainly to influences of metal impurities or residual silanols on the silica gel [19,20]. To prevent the problem, an end-capped reversed-phase C_8 column, LiChrospher 100 RP-8 (e), was used.

The retention decreased with increasing concentration of acetonitrile in the mobile phase, and addition of 2-4% (v/v) of acetic acid in the mobile phase was effective in the prevention of tailing peaks. Although the maximum absorption wavelength of OTC standard solution occurred at both 267 and 354 nm using a photo-diode array detector (see Fig. 2), the wavelength adjusted to 354 nm was used for the specific detection for OTC. The best peak of the target compound was obtained using acetonitrile–acetic acid–water (28:4:68, v/v/v) as the mobile phase.

Fig. 1A shows chromatogram of OTC standard (0.5 μg/ml) obtained under the established con-

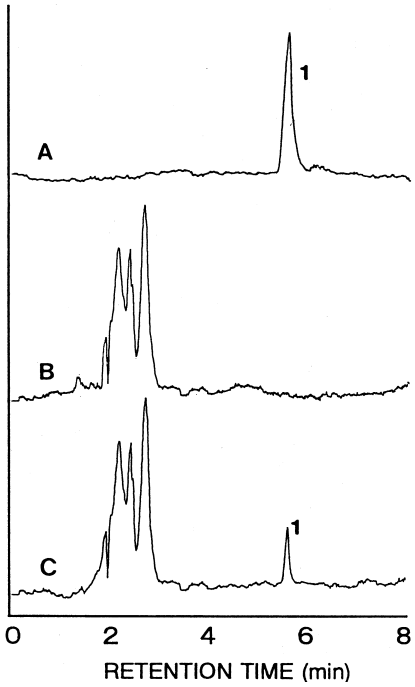

Fig. 1. HPLC chromatograms (photo-diode array detector set at 354 nm). (A) Standard (0.5 μg/ml); (B) blank milk sample; (C) spiked (OTC 0.1 μg/ml) milk sample. Peak 1=oxytetracycline (retention time=5.7 min). For HPLC conditions see Section 3.1.

ditions. The retention time of target compound was 5.7 min.

3.2. Sample preparation

The advantage of the present method is that OTC in milk can be determined using HPLC without complex extraction and clean-up procedures, such as homogenization of the sample with extraction solvent, evaporation of the extracted solution, liquid-liquid partition [8,10,14] and solid-phase extraction using a pre-packed cartridge column [13,19,20]. Namely, the sample (1 ml) preparation was performed with shaking with 1.5 ml of 20% (v/v) TCA solution followed by filtration by 0.45-μm filter unit. Since OTC is decomposed promptly in basic solutions (pH 7.0-8.5) [21,22], the TCA solution, an acid solution, was used in the preparation. Use of 1.5 ml of the above solution, 1.5-times the volume of a milk sample, as a solution for the extraction/deproteinization gave fine recoveries of OTC (Table 1) and the prompt coagulation of protein in the milk. The time required for a sample preparation was less than 3 min.

The resulting extract was free from interference, as can be seen in HPLC traces of blank (Fig. 1B) and spiked (OTC, 0.1 μg/ml) milk sample (Fig. 1C). The spiked level was the MRL in milk. No tetracyclines, like tetracycline, chlortetracycline and doxycycline, showed interference in the OTC peak.

3.3. Calibration, recoveries and identification

The calibration graph was obtained by plotting peak area against amount and was linear over the range 0.2-20 ng. The correlation coefficient, 0.9997, was highly significant ($P<0.01$). The detection limit of OTC was 0.2 ng. Moreover, the precision of the procedure was obtained from relative standard deviation (RSD) of areas calculated for five replicate injections of 1 ng of OTC. The value was 0.8% for OTC.

Table 1 summaries the average recoveries of OTC from milk at three different spiking levels (0.1, 0.5 and 1.0 μg/ml). Satisfactory results were obtained and the average recoveries were greater than 89.8% with coefficients of variation (CVs) between 0.6 and 4.1%. In a practical analysis, the limit of detection (LOD) (signal-to-noise ratio>5) of OTC was 0.05 μg/ml. The value was well below the MRL (0.1 μg/ml). The total time required for the analysis of one sample was below 10 min. The high recovery and low CV together with the economical analysis time indicate that this method has a good precision and may be accurate.

In the present method, the HPLC photo-diode

Table 1
Recoveries of oxytetracycline from milk[a]

Spiked (μg/ml)	Recovery (%)	
	Inter-day	Intra-day
0.1	91.8 (1.6)	89.8 (4.0)
0.5	94.1 (4.1)	92.5 (2.3)
1.0	93.9 (0.6)	91.0 (1.4)

[a] Data are averages. $n=5$; coefficients of variation in parentheses (%).

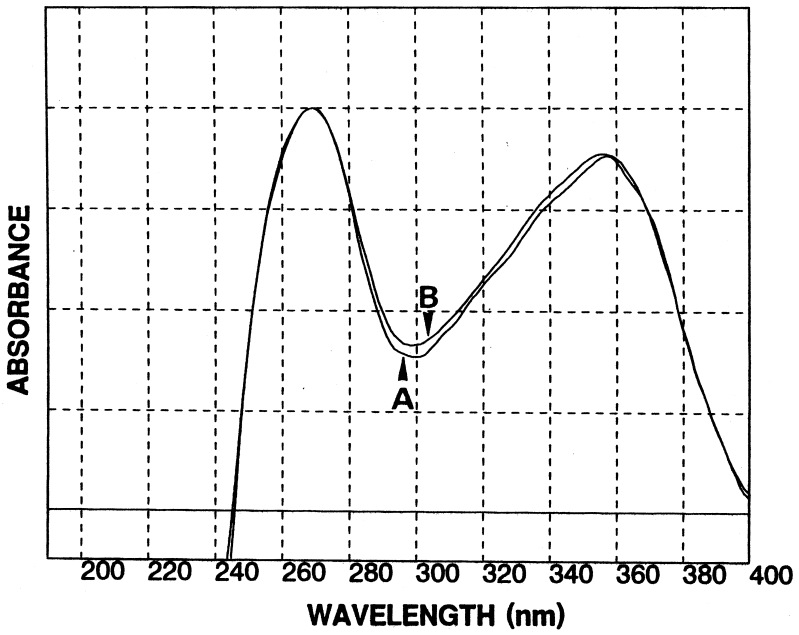

Fig. 2. Normal absorption spectra of peaks at 5.7 min for oxytetracycline in chromatograms (see Fig. 1). (A) Standard; (B) spiked milk extract.

array detector chosen allowed the separation and identification of OTC by its retention time and spectrum. OTC could be identified in sample with by retention time and absorption spectrum. The OTC spectrum obtained from sample is practically identical with that of the standard. A spectra of the OTC peak of a milk obtained with the photo-diode array detector is given in Fig. 2.

3.4. Monitoring residues in marketed milk

Using the present method, 20 different samples of commercial milk that were circulated in Osaka City were analyzed. OTC was not detected in the milk samples. The resulting chromatograms were free from interference (see also Fig. 1).

In conclusion, a rapid and simple analytical method for determination of OTC in milk was developed. Characteristics of this procedure are as follows: (1) simple, direct HPLC analysis; (2) shorter analysis time, total 10 min/sample; (3) highly precise and economical. Therefore, this procedure may be useful for monitoring residues in milk.

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