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# High-throughput analysis of tetracycline and penicillin antibiotics in animal tissues using electrospray tandem mass spectrometry with selected reaction monitoring transition

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**Abstract**

A simple, rapid, and simultaneous analysis method for oxytertracycline, tetracycline, chlortetracycline, penicillin G, ampicillin, and nafcillin in meat has been developed by using electrospray ionization tandem mass spectrometry. The sample preparation was performed by homogenizing with water followed by a centrifugal ultrafiltration, after addition of internal standards (demeclocycline, penicillin G-d5, ampicillin-d5 and nafcillin-d6). The MS/MS analysis involves the combined use of sample enrichment on the short column and a multiple reaction monitoring technique. The overall recoveries from animal (bovine and swine) muscle, kidney, and liver fortified at the levels of 0.05 and 0.1 ppm ranged from 70 to 115% with the coefficients of variation ranging from 0.7 to 14.8% ($n = 5$). Analysis time, including sample preparation and determination, is only 3 h per eight sample and detection limits for all antibiotics are 0.002 ppm. The method is considered to be satisfactory for the rapid screening of the tetracycline and penicillin antibiotic residues in meat.


*Keywords:* Tetracycline; Penicillins; Animal tissues; ESI LC–MS/MS

## 1. Introduction

Tetracycline (TC) and penicillin (PC) antibiotics (Fig. 1) are commonly used all over the world as veterinary medicines and feed additives because of their economical advantages [1,2]. In Japan, oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), ampicillin (ABPC), penicillin G (PCG), and nafcillin (NFPC) are approved to use for domestic animals, and they are often used for treatment of mastitis, pneumonia, bacterial diarrhea, and bacterial arthritis in food-producing animals. Such wide utilization may lead to residue problems in livestock production, therefore, maximum residue limits (MRLs) have been established for OTC, TC, and CTC of 0.2–1.2 μg/g in edible animal tissues being sum of them and for PCG of 0.05 μg/g. For ABPC and NFPC, the MRLs of 0.005–0.3 μg/g are going to be established in edible animal tissues to protect the consumers in Japan. One of the major role as public health agencies is to provide safe products for consumers through quantification of these residues in livestock products.

Microbiological assays have been most commonly used to analyse such residues, but they are complicated, time consuming, and non-specific. In contrast, high-performance liquid chromatography (HPLC) is a fast and reliable technique with high sensitivity, so a number of HPLC methods for TC and PC antibiotics in animal tissues have been reported up to date [3–8], yet the simultaneous multi-residue analysis has not been reported, for some properties of these compounds make the multiresidue analysis difficult, as will be described later. Therefore, we wished to establish a simple, rapid, and simultaneous analysis method of tetracycline and penicillin antibiotics in animal tissues.

TC antibiotics have unfavorable properties for developing the method, such as formation of chelate complex with metal ions and binding silanol groups in the stationary phase [1]. However, as we have previously reported in the analytical method of TC antibiotics in animal tissues, we could solve these problems by adding oxalic acid into the mobile phase of HPLC system and adding ethylenediaminetetraacetic acid sodium salt into the extraction solvent adjusted to pH 4.0 [1,9,10]. On the other hand,

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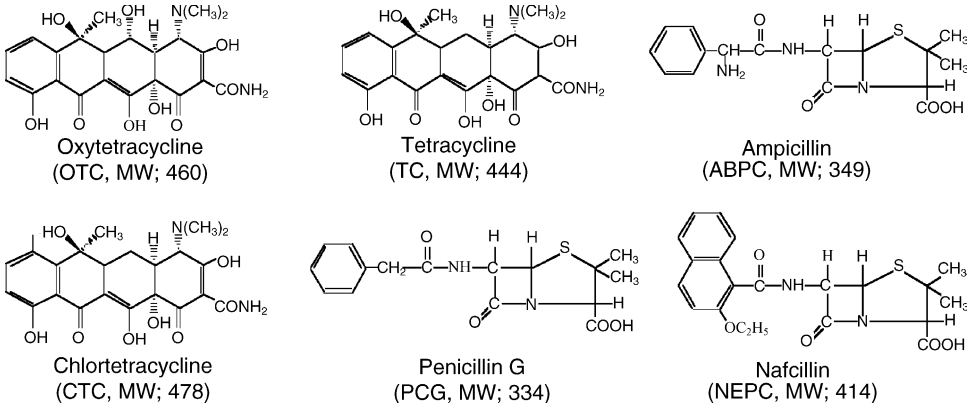


Fig. 1. Tetracycline and penicillin antibiotics.

PC antibiotics decomposes under acidic or basic condition, so we used the neutral mobile phase in LC and purified under the neutral conditions to analyse PC antibiotics [2,11–14]. This makes the simultaneous multiresidue analysis for both antibiotics difficult. Nonetheless, it is possible to establish the multiresidue method if we could obtain the optimal condition by giving a careful thoughts to the chemical properties of the TC and PC antibiotics.

In this paper, we describe in detail the high-throughput simultaneous analysis of TC and PC antibiotics in animal tissues using electrospray tandem mass spectrometry (ESI-MS/MS).

## 2. Experimental

### 2.1. Chemicals and reagents

Methanol, distilled water, and formic acid were of HPLC analysis grade.

Potassium salts of PCG, ABPC, and NFPC were obtained from Sigma (St. Louis, MO, USA) and potassium salts of PCG-d5, ABPC-d5, NFPC-d6 from Hayashi (Osaka, Japan). OTC, TC, CTC, and demeclocycline (DMCTC) were supplied by Pfizer (Tokyo, Japan). Each stock solution of the antibiotics and their internal standards were prepared by dissolving 50 mg in 50 mL of distilled water. They were stored in 10 mL light-resistant vials at 5 °C and were stable for up to 1 week.

Amicon Ultrafree-MC and Microcon YM were purchased from Millipore (Bedford, MA, USA).

### 2.2. Apparatus

The HPLC system consisted of an HP1100 series binary pump, a column compartment and an auto sampler (Hewlett-Packard, Palo Alto, CA, USA). The MS/MS system consisted of a Quattro II triple quadrupole tandem mass spectrometer (Micromass UK, Altrincham, UK) equipped with a Z-spray API source.

### 2.3. Sample enrichment

Sample enrichment was performed on a TSK-Guardgel ODS-80 Ts column (5 μm, 15 mm × 3.2 mm I.D.; Tosoh, Tokyo, Japan) at 30 °C. The mobile phase consisted of a stepwise gradient. Mobile phase A was distilled water containing 0.05% formic acid. Mobile phase B was methanol containing 0.05% formic acid. The flow rate was 0.2 mL/min. The gradient conditions were as follows, base on time ($t$) set at the pump: $t$ = 0.00–0.50 min, hold %B = 0; $t$ = 0.51–6.00, hold %B = 100.

### 2.4. ESI-MS/MS conditions

The desolvation gas (nitrogen) temperature and flow-rate were set at 200 °C and 370 L/h, respectively. The ion source temperature was set at 100 °C. The instrument was operated in the positive and negative ion modes for the tetracyclines and penicillins, respectively, because more abundant ions were observed for tetracyclines in positive mode and for penicillins in negative mode. Collision-induced dissociation was performed using argon as the collision gas at the pressure of $1.9 \times 10^{-3}$ mbar in the collision cell. The other mass spectrometric parameters are summarized in Table 1.

### 2.5. Sample preparation

A 5 g aliquot of a representative sample was weighed into a 50 mL centrifuge tube and was added 0.5 mL of a mixed internal standard solution (0.5 μg/mL of PCG-d5, ABPC-d5, NFPC-d6, and DMCTC aqueous solution). The mixture was blended with 5 mL of ultra pure water for 2 min using a high speed blender. After centrifugation (13,000 rpm, 5 °C, 20 min), a 350 μL aliquot of the supernatant was put into the ultrafilter unit (Ultrafree-MC/PB, NMWL = 10,000) that was prewashed by adding 400 μL each of 1.0% Tween 20 and ultra pure water, and centrifuged (13,000 rpm, 20 °C, 30 min). An 50 μL of the filtrate was injected into the LC–ESI-MS/MS system.

### 2.6. Quantitation

Calibration curves were constructed by peak–area ratios of the antibiotics to internal standards (PCG-d5, ABPC-d5, and NFPC-d6 were used for PCG, ABPC, and NFPC, respectively, and DMCTC was used for OTC, TC, and CTC). Recoveries were