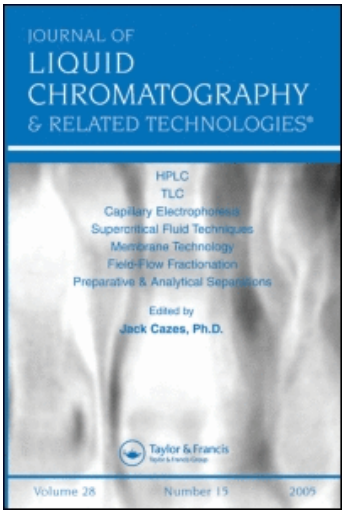


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# Liquid Chromatographic Determination of Oxytetracycline and Chlortetracycline Residues in Animal Tissues

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# LIQUID CHROMATOGRAPHIC DETERMINATION OF OXYTETRACYCLINE AND CHLORTETRACYCLINE RESIDUES IN ANIMAL TISSUES

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## ABSTRACT

A simple and sensitive method has been developed for the simultaneous determination of oxytetracycline and chlortetracycline in animal tissues by liquid chromatography(LC). Tissue samples were extracted with EDTA-McIlvaine buffer(ph4.0)/methanol(3/7). The extracts were purified with Sep-pak C18 and Bond Elut SCX cartridges. Two tetracyclines were separated with a synthetic polymer based-ODS column under basic condition. They were determined by LC with programmable fluorometric detector.

## INTRODUCTION

Oxytetracycline(OTC) and chlortetracycline(CTC) belong to groups of tetracycline antibiotics(TCs) that are used most frequently with respect to veterinary medicine, animal nutrition and feed additives. Therefore, monitoring of residual TCs is important from the viewpoint of veterinary food hygiene. Many methods have been developed for the determination of simultaneous or individual OTC and CTC in biological samples (1-8). These meth-

ods have been based mainly on liquid chromatography(LC) with UV(1-6) or fluorometric(7,8) detection. But they lacked sensitivity required to detect TCs residues and involved tedious manipulations. This paper describes a rapid and sensitive method for the determination of OTC and CTC in tissues by LC with programmable fluorescence detection and ODS based poly(styrene divinylbenzen) copolymer as the stationary phase. This method is suitable for routine analysis of residual OTC and CTC in animal tissues.



## EXPERIMENTAL

### Chemicals

OTC hydrochloride and CTC hydrochloride were kindly donated by Pfizer Pharmaceuticals Inc.(Tokyo,Japan) and Takeda Chemical Industry Ltd.(Osaka,Japan), respectively. Acetonitrile was of LC grade and all other chemicals were of analytical grade from Cica-Merck(Tokyo,Japan). Sep-pak C18 and Bond Elut SCX cartridges were purchased from Waters Association(Milford,MA,U.S.A.) and Analytichem International(Harbor City,CA,U.S.A.),respectively. LC grade water was obtained by purifying reversed osmosis water in a Milli-Q II system(Millipore,Bedford,MA,U.S.A.).

### Apparatus and chromatographic conditions

An LC system consisted of two 6AD pumps, a SIL-6B auto injector, a SCL-6B system controller, a CTO-6A column oven, a RF-550A spectrofluorometer, a C-R4AX integrator(Shimadzu,Kyoto,Japan) and a KT-35 degasser(Shodex,Tokyo,Japan). All analyses were carried out using a ODP-50 5-um 250mm x 4.6mm(I.D.)column, which was purchased from Asahi chemical ind.(Tokyo,Japan). The mobile

phase was prepared by mixing 900 ml of Sorensen buffer(pH12.0) and 100 ml of acetonitrile. The flow rate was 1ml/min and the column temperature was maintained at 40°C. The wave length of spectrofluorometer was changed to ex. 350nm, em. 420nm from ex.374nm,em.508nm 7 min later, by using time programming technique.

Sample preparation procedure

Minced 10g tissue was homogenized with 30ml of 10mM $Na_2EDTA$-McIlvaine buffer(pH 4.0)-methanol(3:7) mixture and centrifuged twice. The supernate was combined and concentrated to ca.5 ml under vacuum. The concentrated solution was offered onto a Sep-pak C18 cartridge column. The cartridge was rinsed with 5 ml of water and eluted with 10 ml of methanol. The eluate was offered onto the next Bond Elut SCX cartridge. The cartridge was rinsed with 10 ml of water and eluted with 10 ml of 1N HCl-methanol(2:8). The eluate was adjusted to pH 12 with 10N sodium hydroxide and injected to the LC after an hour.



## RESULTS AND DISCUSSION

Variation of the emission spectra of OTC(I) and CTC(II) for different pH(9-12) solution are shown in Fig.1. The maximum fluorescence wave length for OTC is at 390nm on excitation, and 495-500nm on emission, and that for CTC is 350nm on excitation, and 405-410nm on emission,respectively. As CTC decomposes in alkaline medium to form isochlortetracycline(ISOCTC)(7), the fluorescence intensities of TCs are strongly dependent on the pH of the solution. Perhaps, the same phenomenon may happen on OTC to that on as CTC. The reduced fluorescence intensities in alkaline medium are due to the decomposition of these two TCs.

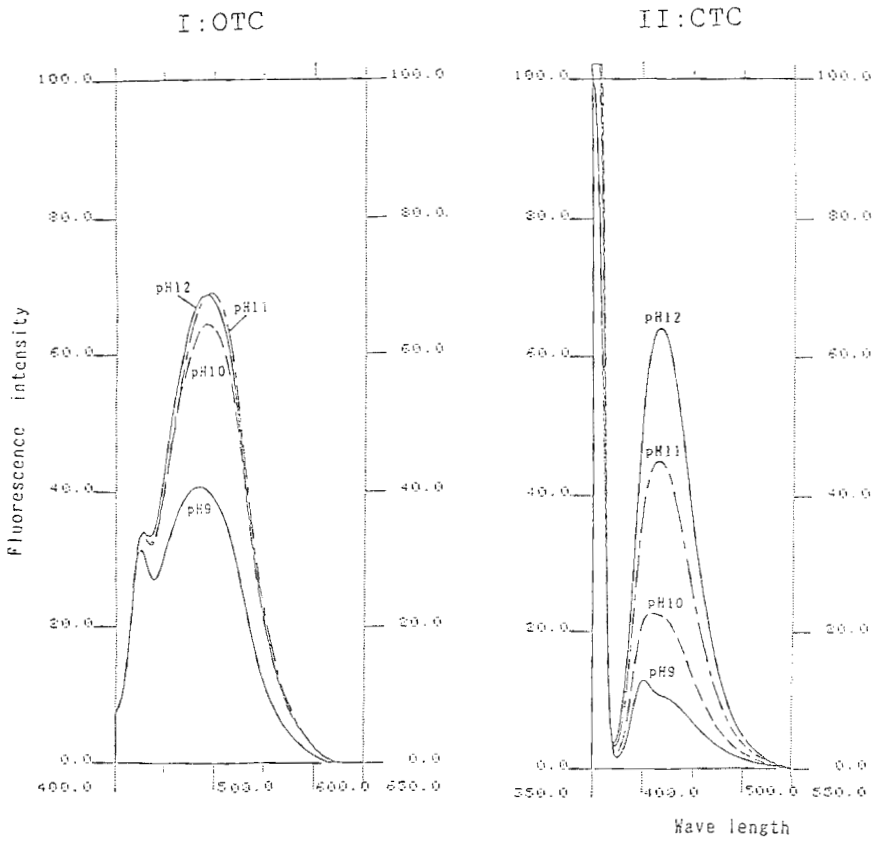


Fig. 1. Emission spectra of TCs (I:OTC, II:CTC) for different PH(9-12) solutions.

The time-dependent changes of decomposed TCs are shown in Fig.2,3. The fluorescence intensity of OTC reached the maximum value immediately and was held constant for long time. That of CTC increased slowly and reached plateau after an hour, to maintain its intensity for moreover 24 hrs. The reacted time was of one hour was necessary for the simultaneous determination of these two TCs.

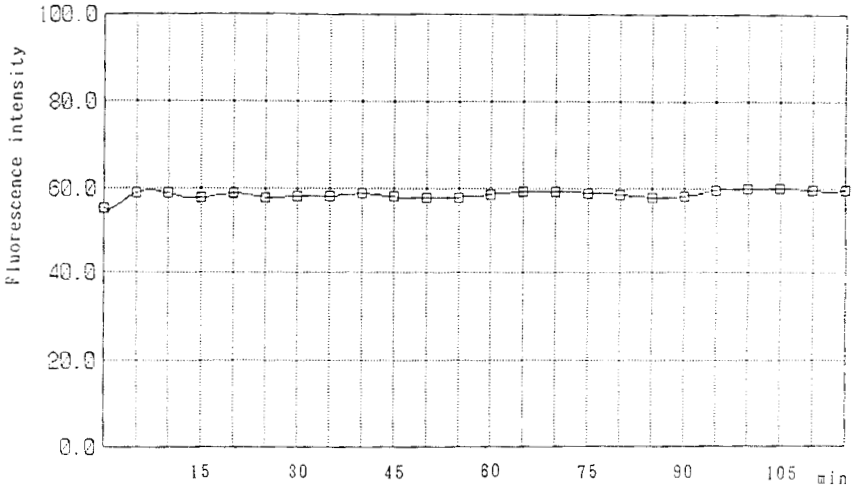


Fig.2. Fluorescence intensity versus time profile of OTC in basic condition(mobile phase).

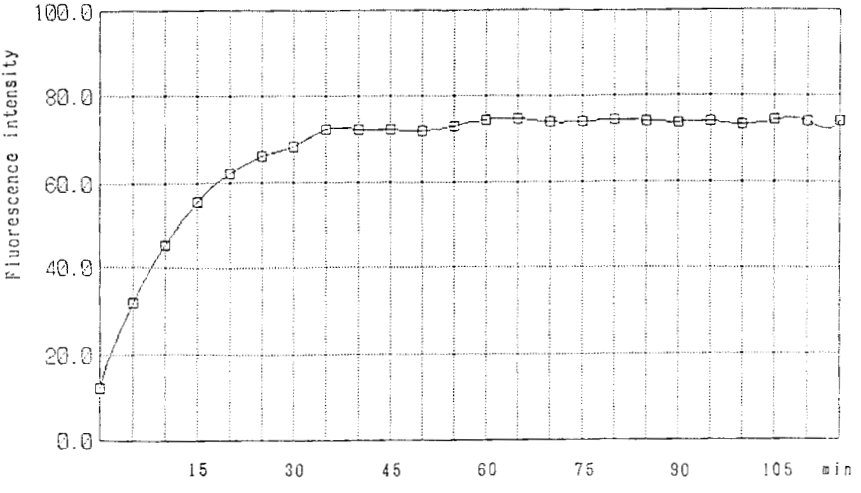


Fig.3. Fluorescence intensity versus time profile of CTC in basic condition(mobile phase).

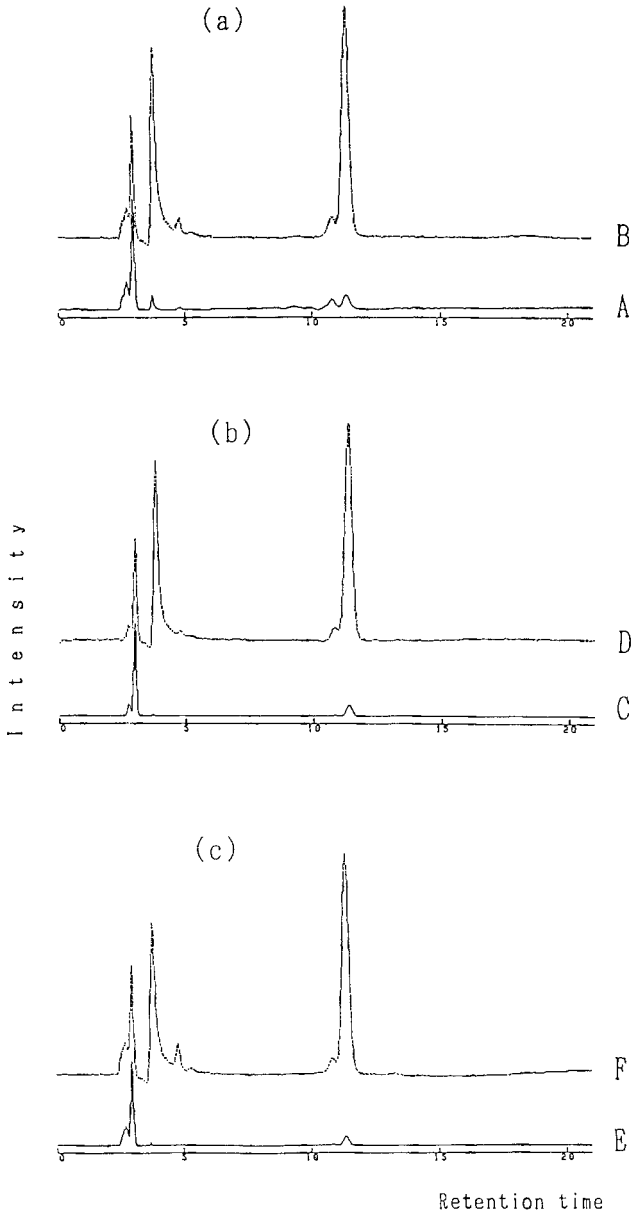


Fig. 4. Liquid chromatograms of drug free & fortified samples; A, C, E:drug free samples; B, D, F:fortified samples; (a):bovine; (b):swine;(c):chicken; LC conditions are described in literature.

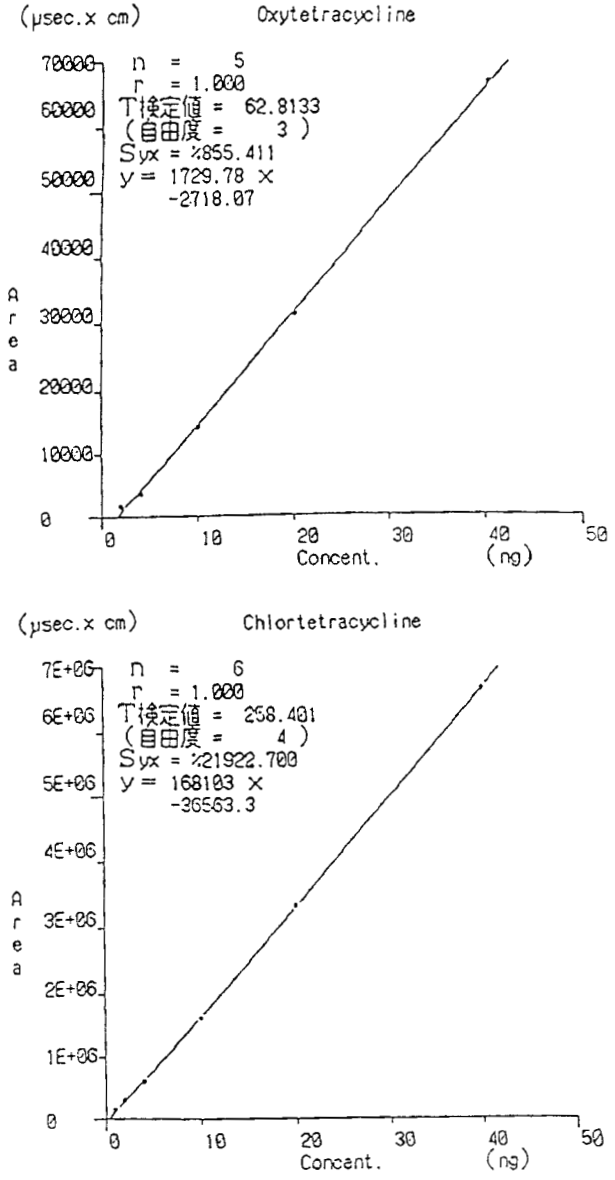


Fig. 5. Calibration curves for OTC and CTC.

Table 1 Recoveries of OTC and CTC from Bovine, Swine and Chicken.

| | Added(μg/g) | Recovery±S.D., C.V.(%) | | |
|---|---|---|---|---|
| | | Bovine | Swine | Chicken |
| OTC | 0.2 | 79.5±5.4, 6.8 | 74.4±4.3, 5.8 | 63.4±1.6, 2.5 |
| | 1.0 | 81.3±7.4, 9.1 | 85.6±3.9, 4.6 | 74.8±4.6, 6.1 |
| CTC | 0.2 | 78.5±6.1, 7.8 | 72.3±6.3, 8.7 | 61.8±3.6, 5.8 |
| | 1.0 | 86.5±9.7, 11.2 | 73.1±6.0, 8.2 | 70.5±4.5, 6.4 |

n=5



Some large interfering peaks derived from the matrix appeared before OTC and CTC peaks in the case of using an only ODS cartridge. The second cartridge of SCX was therefore connected with the ODS cartridge to remove the interfering peaks. Although they still appeared after the use of double cartridges, this method was fairly effective in minimizing the interference with co-extracted components of the meat. Both peak height of 5ng OTC 1ng CTC were more than 4 times that of the interfering peaks. Consequently, the matrix peaks were estimated to be negligible. These results are shown in Fig.4.

Results of calibration runs for the validation range of 2-40ng (OTC) and 1-40ng(CTC), respectively seen in Fig.5, showed excellent linearity (r=0.999).

Two amounts(0.2&1ug) of TCs were added to the drug free minced meat(10g), and their recoveries were measured by the procedure described above. The results are summarized in Table 1, which shows recovery ranges from 61.8 to 86.5% and coefficients of variation (C.V.) from 2.5 to 11.2%.

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