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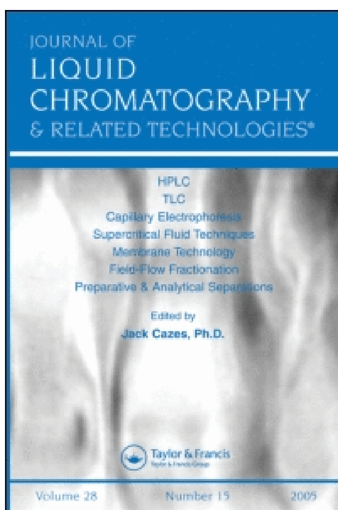

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SIMULTANEOUS DETERMINATION OF THIAMPHENICOL, FLORFENICOL, AND CHLORAMPHENICOL RESIDUES IN MUSCLES OF ANIMALS AND CULTURED FISH BY LIQUID CHROMATOGRAPHY

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ABSTRACT

A liquid chromatographic (HPLC) method for simultaneous determination of thiamphenicol, florfenicol and chloramphenicol residues in muscles of animals and cultured fishes was developed. The drugs are extracted from minced muscles with ethyl acetate, and the extract is evaporated to dryness. The residue is dissolved with 3% NaCl and partitioned with n-hexane. The drugs are extracted with ethyl acetate, and after evaporation of the solvent, the residue is cleaned up by Sep-Pak Florisil cartridge. HPLC analysis is carried out on Chromatorex ODS column, and the drugs are quantitated by UV detector at 225 or 270 nm. The average recoveries of thiamphenicol, florfenicol and chloramphenicol added to muscles at 0.1 ppm were more than 74.1%. The detection limit was 1 ng for each drug standard, which corresponded to 0.01 ppm in muscles.

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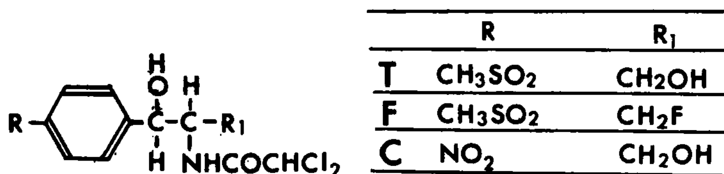

	R	R_1
T	CH_3SO_2	CH_2OH
F	CH_3SO_2	CH_2F
C	NO_2	CH_2OH

FIGURE 1. Chemical Structures of Thiamphenicol, Florfenicol and Chloramphenicol. T:thiamphenicol, F:florfenicol, C:chloramphenicol

INTRODUCTION

Thiamphenicol, florfenicol and chloramphenicol are synthetic antibiotics with similar broad spectrum of activity. As shown in Figure 1, their chemical structure are quite similar. These three drugs were proved to be valuable for the treatment of bacterial infections and are administered to animals for disease prevention (1).

The liquid chromatographic (HPLC) methods to determine thiamphenicol in biological fluids (2), in bovine plasma (3) and in chicken meats (4) are existing, and the detection limits of theirs are 0.5, 0.1 and 0.05 ppm, respectively. HPLC methods to determine chloramphenicol in serum (5,7,9,11) and in tissues (6,8,10,12, 13) have been developed with the detection limits from 0.01 to 7 ppm. As for florfenicol, which has been recently developed in Japan, any determination methods have not yet been published.

In the present study, an investigation to establish a convenient method was conducted successfully for

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the simultaneous determination of thiamphenicol, florfenicol and chloramphenicol residues in muscles of the calf, swine, chicken and cultured fish as sensitive as 0.01 ppm using HPLC.

MATERIALS AND METHODS

Reagents and chemicals

Drugs used in this study were thiamphenicol, chloramphenicol (Sigma Chemical Company, St.Louis, Mo, U.S.A.) and florfenicol (Takeda Yakuhin Kogyo, Co., Tokyo, Japan). Each stock solution was prepared by dissolving 10 mg of each drug in 100 ml of methanol at 100 μg/ml. The working standard solution was prepared at 1 μg/ml in methanol using each 1 ml stock solution. All chemicals were analytical grade (Wako Pure Chemical Industry Ltd, Osaka, Japan). Sep-Pak Florisil cartridge was preconditioned with 5 ml n-hexane followed by 5 ml ethyl ether prior to use.

Apparatus

The HPLC system consisted of a Model 801-SC system controller equipped with a Model 880-PU pump, a Model 850-AS automatic sampler with 20 μl sampling loop, a Model 875-UV ultraviolet spectrometer, a Model 860-CO column oven (Japan Spectroscopic Co., Ltd., Tokyo, Japan) and a Model CR-6A Chromatopak integrator (Shimadzu Sheisakusho Co., Kyoto, Japan). Chromatorex ODS stainless steel, 150 mm X 4.6 mm id (5 μm) (Fuji

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Davison Chemical Ltd., Nagoya, Japan) was used as an analytical column. The flow rate of mobile phase, methanol-water (15:85), was 1.2 ml/min and the column temperature was maintained at 55°C. Thiamphenicol and florfenicol were detected at 225 nm and chloramphenicol at 270 nm.

Extraction and Cleanup

10 g minced muscle were accurately weighed, homogenized 3 min at maximum speed with 50 ml ethyl acetate, centrifuged at 2300 g for 10 min and supernatant was transferred to a round-bottom flask. The residue was homogenized with another 50 ml ethyl acetate, the above procedure was repeated, and the supernatant was collected in the round-bottom flask. The extract was concentrated to 1-2 ml under vacuum on rotary evaporator at 65°C.

The residue was dissolved with 5 ml of 3% sodium chloride solution in an ultrasonic bath and transferred to a separatory funnel with two 10 ml portions of the same solvent. 25 ml n-hexane were added to the separatory funnel, shaken gently, allowed to stand until the two layers separated, and the upper layer was discarded. Another 25 ml n-hexane was added to the separatory funnel, shaken vigorously for 5 min and allowed to stand until layers separated. The lower phase was transferred to another separatory funnel, 40 ml ethyl

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acetate was added and shaken for 5 min. The upper phase was transferred to a flask and the above procedure was repeated with another 40 ml ethyl acetate.

The ethyl acetate layers were collected in the flask and evaporated to dryness under vacuum on a rotary evaporator at 65°C. The residue was dissolved with 5 ml n-hexane in an ultrasonic bath and poured into a Sep-Pak Florisil cartridge and the cartridge was washed with the n-hexane. The flask was rinsed with 5 ml of ethyl ether, the rinsing fluid was poured into the cartridge, and the cartridge was washed as described the above. The drugs were eluted with 5 ml of ethyl ether-methanol (7:3) solution and the eluate was evaporated to dryness under vacuum on a rotary evaporator at 45°C. The residue was dissolved in 1 ml methanol, filtered through a filter membrane at 0.5 μm porosity and applied to HPLC instrument.

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RESULTS AND DISCUSSION

To extract thiamphenicol or chloramphenicol from samples, ethyl acetate (3,4,6,8,11), methanol (2) and water (12,13) have been hitherto used. In the present study, ethyl acetate was used to have clearer extracts. In order to remove lipid by partitioning with n-hexane, the extract was evaporated to dryness and the residue was dissolved with 3% sodium chloride solution. The extract of yellow tail muscle could not be evaporated

to dryness completely because of the 1-2 ml remainder of oily matter.

To remove water-soluble interferings, the aqueous solution was partitioned with ethyl acetate. To select suitable extractant, 5 μg of each drug, i.e. 0.5 ml of each standard solution (10 μg/ml), was added to each of six separatory funnels with 25 ml 10% sodium chloride solution. Each solution was partitioned with one of six kinds of 25 ml solvents, i.e. chloroform, dichloromethane, carbon tetra chloride, ethyl acetate, diethyl ether or petroleum ether. As shown in Table 1, only ethyl acetate was found to extract three drugs simultaneously and most effectively than the other solvents.

This ethyl acetate extract showed highly polar material in the chromatogram. To remove interfering substance, an attempt was made for purification on Sep-Pak Florisil cartridge. The drugs, however, were not eluted from the cartridge with n-hexane or ethyl ether solely. When small amount of methanol was added to ethyl ether, the drugs were eluted from the cartridge to some extent. Finally, the elution of almost all drugs from the cartridge was successful with 5 ml of ethyl ether-methanol (7:3) solution.

Appropriate conditions on mobile phase and wavelength for chromatographic detection were investigated. When acetonitrile-water was used in the present study,

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TABLE 1

Recovery of the Drugs Extracted with Six Different Solvents

Solvent	Thiamphenicol	Florfenicol	Chloramphenicol
$CHCl_3$	0	64	38
CH_2Cl_2	0	83	57
CCl_4	0	0	0
Ethyl acetate	65	92	81
Diethyl ether	0	59	76
Petroleum ether	0	6	24

(%), $CHCl_3$:chloroform, CH_2Cl_2:dichloromethane, CCl_4:carbon tetra chloride, Six 10% NaCl solutions containing 5 μg of each drug were extracted with each solvent.

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the sample solution gave interference peaks in chromatogram, especially around the thiamphenicol peak. Therefore, methanol-water was selected as a mobile phase. As shown in Figure 2, by this mobile phase, the maximum peak absorbance of thiamphenicol and florfenicol was not observed but that of chloramphenicol was at 270 nm. Chloramphenicol was more sensitive at 270 nm than at 225 nm. Therefore, thiamphenicol and florfenicol were detected at 225 nm and chloramphenicol at 270 nm.

As can be seen in chromatograms of muscle extracts in Figure 3, the retention times of thiamphenicol, florfenicol and chloramphenicol were 7.2 min, 14.0 min and 26 min, respectively. Any other interfering peaks were not observed in muscle extract chromatograms. Other drugs such as sulfonamides and fulazoridon, which

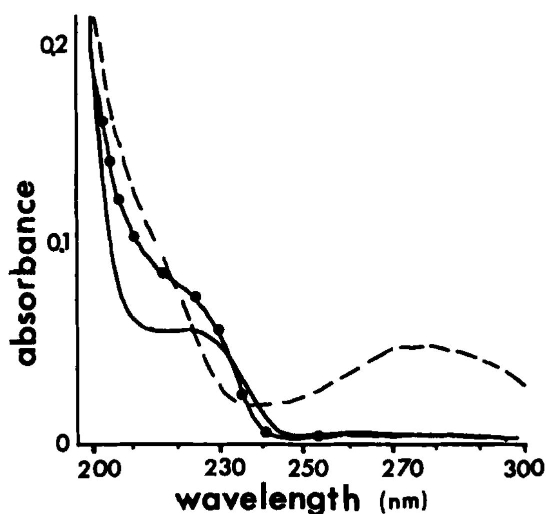

FIGURE 2. Absorption Spectra of Thiamphenicol, Florfenicol and Chloramphenicol in HPLC Mobile Phase. ——:thiamphenicol, •-•:florfenicol, - -:chloramphenicol(each 1.5 μg/ml)

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TABLE 2

Recovery Study of the Drugs from Fortified Muscles

Sample		Thiamphenicol	Florfenicol	Chloramphenicol
Chicken muscles	Rec.,(%)	73.4	81.5	74.4
	SD	0.048	0.032	0.076
	CV (%)	6.4	3.9	10.1
Swine muscles	Rec.,(%)	73.2	80.2	78.7
	SD	0.015	0.012	0.068
	CV (%)	1.9	1.4	8.5
Calf muscles	Rec.,(%)	67.6	74.9	71.4
	SD	0.045	0.058	0.069
	CV (%)	6.6	7.7	9.6
Yellow tail muscles	Rec.,(%)	75.4	79.1	82.6
	SD	0.039	0.021	0.038
	CV (%)	5.2	2.6	4.6

(n=4), 1 μg of each drug was added to muscles.

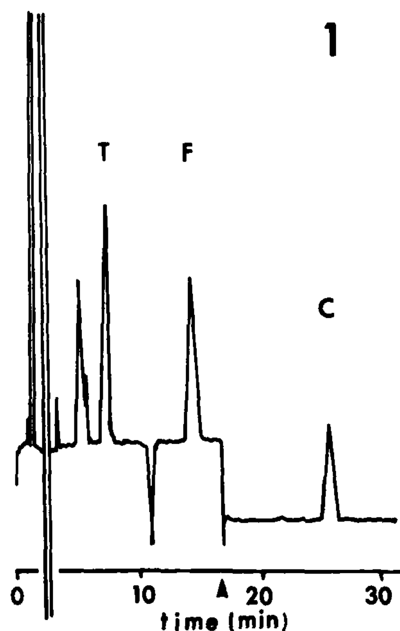

FIGURE 3. Liquid Chromatograms of Standards and Muscle Extracts. 1:standards, T:thiamphenicol, F:florfenicol, C:chloramphenicol,(each 10ng) 2:calf meat extract, 3:chicken meat extract, 4:swine meat extract, 5:yellow tail extract, ▲:wavelength was switched from 225nm to 270nm.

(continued)

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are often used to animals, did not interfere the determination of this method. The standard curve for thiamphenicol, florfenicol and chloramphenicol were as follows;

$X = 2.3586\ Y + 0.0699$, $r = 0.9963$ in thiamphenicol,

$X = 3.0656\ Y + 0.0014$, $r = 0.9968$ in frolfenicol,

$X = 3.9325\ Y + 0.0537$, $r = 0.9996$ in chloramphenicol,

where Y = peak area of drug, X = concentration (μg/ml) of drug and r = correlation coefficient. The standard

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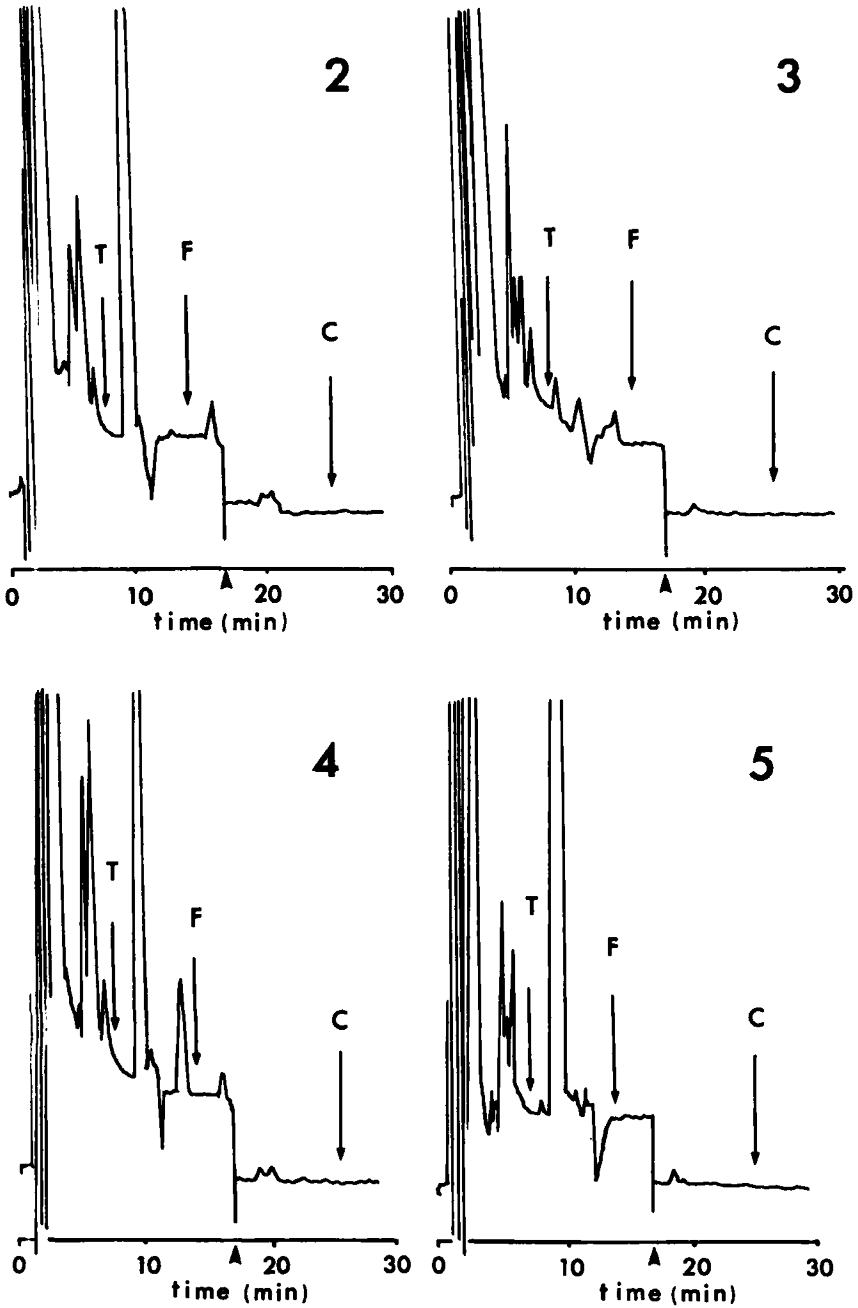

FIGURE 3 (continued)

curve was liner over the range of 1-10 ng in thiamphenicol, 1-20 ng in florfenicol and 1-40 ng in chloramphenicol.

Recovery studies were performed by adding 1 ml of the working standard solutions to 10 g of minced muscles of calf, chicken, swine and yellow tail. As shown in Table 2, the recoveries of drug were more than 71.4%, except 67.6% at thiamphenicol in calf muscles. The detection limit was 1 ng, (5-fold noise level), which corresponded to 0.01 ppm thiamphenicol, florfenicol and chloramphenicol in muscles.

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REFERENCES

1. Azechi,H., "General Survey of Veterinary Medicine", Azechi,H. ed., Japan Veterinary Pharmaceutical Center, Tokyo, Japan (1991) (Japanese text)

2. Meulemans,A., Manuel,C., Mohler, J., Roncoroni,A. and Vulpillat, M., J.Liq.Chromatogr., 4 (1981) 145

3. Felice, L., Abdennebi, E.H. and Ashraf, M, J.Assoc.Off.Anal. Chem., 71 (1988) 1156

4. Nagata, T., Saeki, M., J.Chromatogr., 565 (1991) 471

5. Wiese, B., Martin, K., Chromatographia, 15 (1982) 737

6. Johannes, B., Korfer, K.H., Schad,J., and Ulbrich, I., Arch. Lebensmittelhyg., 34 (1983) 1

7. Van Lee, J.J., Rijsbergen, H.B.J., Tjaden,U.R. and Bennekom,W.P., Anal. Chim. Acta, 149 (1983) 29

8. Haagsma, N., Schreuder,C. and Rensen, E.R.A., J.Chromatogr. 363 (1986) 353

9. Tyczkowska, K., Hedeen, K.M., Aucoin,D.P. and Aronson,A.L., J.Chromatogr.Sci., 26 (1988) 533

10 Tjaden,U.R., Stegehuis, D.S., Reeuwijk, B.J.E.M., Lingeman,H. and Greef, J.D., Analyst, 113 (1988) 171

11 Wong, S.H.Y., Cudny, B., Aziz, O., Marzouk.N. and Sheehan, S.R., J.Liq.Chromatogr., 11 (1988) 1143

12 Aerts, R.M.L., Keukens,H.J.K. and Werdmuller, G.A., J.Assoc.Off.Anal.Chem., 72 (1989) 570

13. Ashton, M., J.Liquid.Chromatogr., 12 (1989) 1719

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