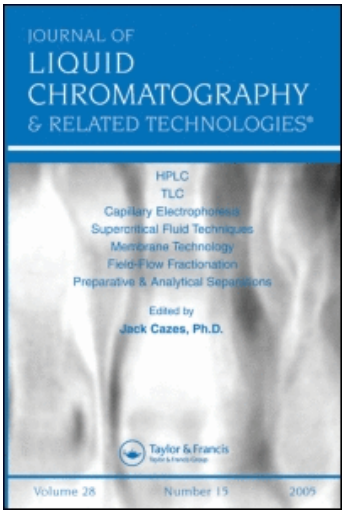

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF LASALOCID SODIUM IN CHICKEN TISSUE

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method with fluorometric detection has been developed to determine lasalocid sodium(Fig.1) residues in chicken tissues. Lasalocid sodium was extracted from tissues by homogenizing them with methanol, purified by silicagel cartridge column and separated by HPLC using an ODS column.

INTRODUCTION

Lasalocid belongs to the group of carboxylic polyether antibiotics that are produced by Streptomyces lasaliensis, and acts as a ionophore. Lasolocid has been recognized to complex a wide variety of cations including alkali-metals, alkaline-earth -metals, transition-metals and heavy metals and lanthanides(1).

In Japan, Lasalocid is added in the form of the sodium salt to the chick and to the broiler chicken feeds as a coccidiostat at levels of 75 g/ton. Moreover, it is used as a growth promoter in cattle feeds in many countries except Japan.

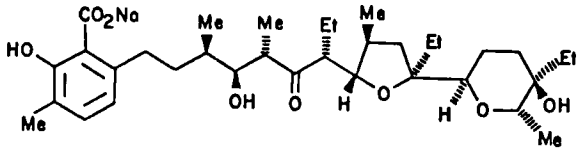

Fig. 1. Structure of lasalocid sodium

Lasalocid sodium is non-volatile and does not absorb light in the UV-visible region. These properties of lasalocid sodium restrict a number of chemical techniques that are available for its determination in chicken tissues. However, lasalocid sodium exhibits fluorescence with excitation at 308-315 nm and emission at 400-430 nm, depending upon the solution(2).

A review of analytical methods for lasalocid had been published by Weiss and MacDonald(3). According to this review, analytical investigations of lasalocid include determination for pyrolysis GC(4), pyrolysis GC-MS(5), bioassay(6,7) and normal-phase HPLC (2,8,9). And another methods of determination for residual lasalocid in tissues, fluid and feed are spectrofluorometry(10,11), HPLC-fluorescence derivatization(12) and HPTLC(13). These methods, however, are tedious, non-specific or time-consuming.

In this paper, we describe a reversed-phase and underivatived fluorometric HPLC method for the determination of lasalocid in chicken tissues.

EXPERIMENTAL

Reagents and chemicals

Lasalocid sodium was obtained from Sigma(St.Louis,MO,U.S.A.).

A stock solution was prepared by dissolving 100 mg of lasalocid sodium in 100 ml of methanol(1000 μg/ml). Spiking solutions were prepared from the stock solution(1-10 μg/ml). Working solutions

for HPLC were prepared by diluting the stock solution with acetic acid:methanol(1:99,v/v, 1-10 μg/ml). Sep-pak silica cartridge was purchased from Waters (Milford, MS,U.S.A.). Acetonitrile was of HPLC grade(Cica-Merck, Tokyo,Japan). All other chemicals used were analytical grade and were obtained from Wako(Osaka,Japan).

Apparatus and HPLC conditions

The HPLC system consisted of a 880 pump, a Tu-100 column oven, an AS-L 350 auto sampler(JASCO,Tokyo,Japan), an F-1000 fluorometric detector(Hitachi,Tokyo,Japan) and a CR-6A integrator(Shimadzu, Kyoto,Japan). A spectrofluorophotometer was used an RF-540 (Shimazdu).

The analytical column (250 mm x 4.6 mm I.D.) was packed with a 5 μm Nucleosil 100 C18 (Macherey-Nagel, Düren, West Germany) and maintained at 40°C. The mobile phase consisted of 20 mM phosphate buffer(pH7.0):acetonitrile:methanol(25:35:40,v/v/v). The flow-rate was 1.0 ml/min. The fluorescence detector was adjusted for an excitation wavelength of 310 nm and an emission wavelength of 420 nm.

Sample preparation

Liver tissue. A 5 g of minced tissues was homogenized with 20 ml of methanol. After the centrifugation at 3000 rpm(2300 g) for 10 min, 20 ml of methanol was added to the residue and the same procedure was repeated. To the combined supernate was added 80 ml of water, and the mixture was extracted with 60 ml of carbon tetrachloride, twice. The combined lower layer was concentrated to ca. 5 ml and transferred to the Sep-pak silica cartridge. The cartridge was rinsed with 5 ml of methylene chloride, and eluted with 10 ml of acetic acid:methanol(1:99,v/v). The eluate was evaporated and dissolved with 2 ml of methanol. The methanol

solution was filtered through a micropore filter if necessary. A 10-40 μl aliquot was injected into the HPLC system.

Muscle and gizzard tissue Lasalocid was extracted with 20 ml of methanol similarity to the case of liver tissues. That is, the methanol solution was directly concentrated under vacuum and followed to the same procedure as in liver tissue preparation.

RESULTS and DISCUSSION

Fig. 2 shows the excitation and emission spectra of lasalocid sodium in mobile phase. The maximum wavelength of excitation spectrum is at 310 nm and that of the emission spectrum at 420 nm. By testing several types of cartridge columns, i.e., alumina(acid, neutral and basic), florisil and ODS, it was found that silica was satisfactory for separating lasalocid sodium from interferential matrix.

Lasalocid sodium loaded on cartridge was eluted with acetic acid:methanol(1:99,v/v) solution, which more effective than methanol alone. Recoveries of lasalocid sodium from the cartridge were 98+2% for 7 ml of acetic acid:methanol(1:99,v/v)solution and 93+5% for 20 ml of methanol.

Before transferring into the cartridge , liquid/liquid partition was required for removing impurities of liver tissues. This procedure was not necessary in muscle and gizzard tissues.

Fig. 3 shows typical liquid-chromatograms of lasalocid sodium in chicken tissues. All interfering peaks were detected within the first five min, and no interfering peaks were observed when the other ionophores (salinomycin and monensin) and tetracyclines · (oxy- and chlor-) were fortified at chicken tissues. Therefore

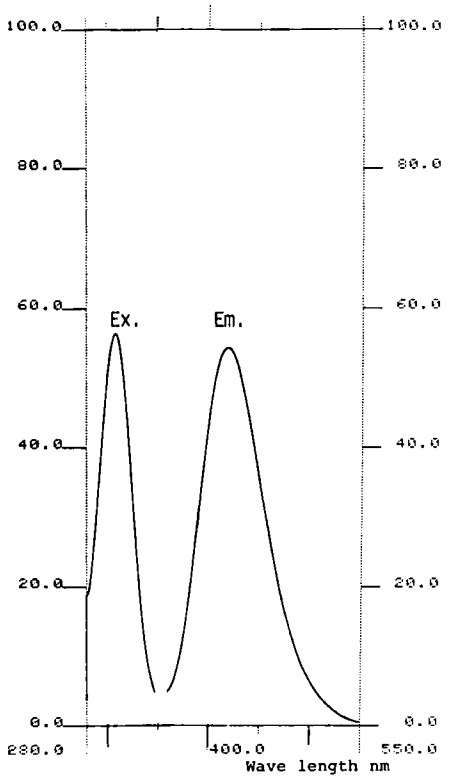

Fig. 2. Excitation(left)-emission(right) spectra of lasalocid sodium(1 ppm, HPLC st. in mobile phase)

chicken samples were able to inject continuously at 15 min intervals by autosampler.

When we used 20mM phosphate buffer(pH7.0):acetonitrile(25:75,v/v) as a mobile phase, peak shape of lasalocid was tailing. But symmetric peak of lasalocid has been obtained when about half volume of methanol instead of acetonitrile was added as the mobile phase(Fig.3).

In Table 1, is summarized the recovery on chicken tissues spiked with lasalocid sodium at the concentrations of 0.4 and 2 ppm.

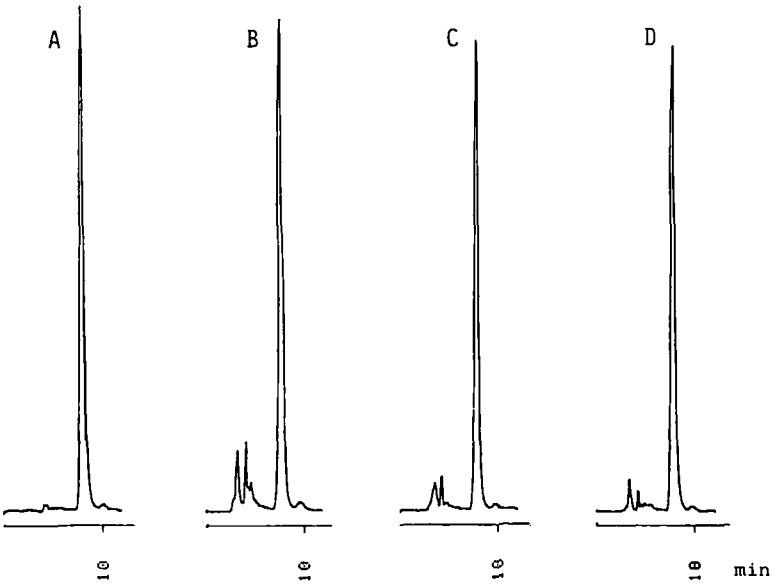

Fig. 3. Typical chromatograms of lasalocid sodium (10 ng)
A; standard, B; chicken muscle spiked st.,
C; chicken gizzard spiked st., D; chicken liver spiked st.

Table 1. Recoveries of lasalocid sodium from chicken tissues

Tissues	Added (μg/g)	Mean±S.D.(%)
Muscle	0.4	99.6±3.1
	2.0	100.5±1.5
Gizzard	0.4	94.0±3.6
	2.0	98.2±2.3
Liver	0.4	86.2±4.2
	2.0	96.0±3.7

n = 5

Five samples of the ground tissues were spiked at each levels. The recoveries ranged from 86.2 to 100.5 % with a corresponding standard deviation range of 1.5 to 4.2 %.

The calibration curve was linear between 1 ng and 500 ng(r=0.996). The quantitation limit was 0.01 ppm. And the detection limit was 0.5 ng (s/n=2).

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