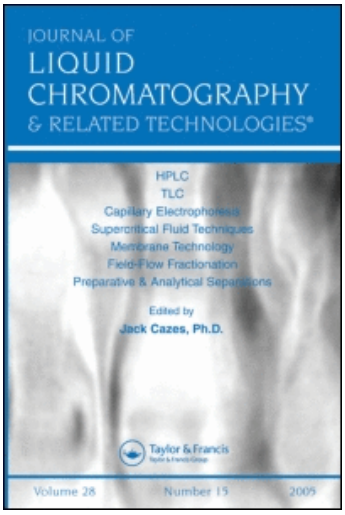

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Determination of Tylosin in Bovine Muscle, Kidney and Liver

Lily J. -Y. Keng[a]; Joe O. Boison[a]
[a] Health of Animals Laboratory Food Production and Inspection Branch, Saskatoon, Saskatchewan, Canada

To cite this Article Keng, Lily J. -Y. and Boison, Joe O.(1992) 'High Performance Liquid Chromatographic Determination of Tylosin in Bovine Muscle, Kidney and Liver', Journal of Liquid Chromatography & Related Technologies, 15: 12, 2025 — 2034
To link to this Article: DOI: 10.1080/10826079208016322
URL: http://dx.doi.org/10.1080/10826079208016322

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(12), 2025-2034 (1992)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF TYLOSIN IN BOVINE MUSCLE, KIDNEY AND LIVER

LILY J.-Y. KENG AND JOE O. BOISON*
Health of Animals Laboratory
Food Production and Inspection Branch
Agriculture Canada
116 Veterinary Road
Saskatoon, Saskatchewan S7N 2R3
Canada

ABSTRACT

A method is described for the determination of the residual macrolide antibiotic, tylosin, in bovine muscle, kidney, and liver by high performance liquid chromatography. Tylosin was extracted from the tissues with dichloromethane, concentrated on a silica gel solid phase extraction cartridge, and analysed using a reversed-phase C18 column, with UV detection at 280 nm and a mobile phase of 0.005 M ammonium dihydrogen phosphate-acetonitrile-methanol (8:72:20) (v/v/v). The method, which has a detection limit of 15 ng/g in kidney, liver, and muscle, was validated by demonstrating that it is accurate, precise, and reproducible and provides high recovery from tissues fortified with tylosin.

* **Author to whom correspondence and reprint requests should be directed**

INTRODUCTION

Tylosin, a macrolide antibiotic, is used as a feed additive in chickens, swine, and beef cattle for growth promotion. It is also used for the treatment and prevention of swine dysentery, and to reduce the incidence of liver abscesses in beef cattle. Tylosin residues in tissues of food-producing animals can have toxic effects on humans that may include allergic reactions of consumers and induction of resistant bacteria (1).

Most methods available for the determination of tylosin use microbiological assays (2, 3). Although these methods are suitable for screening drug residues, they are inadequate for quantifying tylosin at the official Canadian tolerance level of 0.2 ppm (4) in edible tissues. Other chromatographic methods including thin layer chromatography (TLC), gas chromatography (GC), and liquid chromatography (LC), have been described (1, 5, 6, 7) for the determination of tylosin. However, only a few of the reported methods have been applied to residue analysis in animal tissues (1, 2, 5, 6, 7). For these methods, the detection limits have either been slightly below or above the official tolerance level for tylosin in animal tissues. In addition, these methods usually involved cumbersome and lengthy procedures making them unsuitable for routine laboratory analysis of tylosin. A simpler, more sensitive, reproducible, and accurate procedure is therefore required for the quantitative laboratory

analysis of tylosin residues in tissues of food-producing animals.

This paper describes a simple, rapid, reproducible, and sensitive HPLC method developed for the quantitative analysis of tylosin in bovine muscle, kidney, and liver.

MATERIALS AND METHODS

Reagents

Tylosin tartrate (containing 949 μg tylosin per mg of tylosin tartrate) was purchased from Sigma Chemical Company (St. Louis, Missouri, U.S.A.). Ammonium dihydrogen orthophosphate was obtained from BDH (Toronto, Ontario, Canada). All other reagents were of HPLC grade.

Materials

Silica gel solid phase extraction cartridges (J. T. Baker, 500 mg/6 ml capacity) were purchased from John's Scientific Inc. (Toronto, Ontario, Canada).

HPLC Equipment

The HPLC system included a Waters 600E System Controller, a Waters 610 Fluid Unit, a 700 Autosampler (Waters Chromatography Division, Mississauga, Ontario, Canada), and a Kratos 783 Variable UV detector (Kratos Analytical, Ramsey, New Jersey, U.S.A.). The detector was set at a wavelength of 280 nm and a sensitivity setting of 0.003 AUFS. The column (25 cm x 4.6 mm

I.D., Phenomenex, Torrance, California, U.S.A.) was prepacked with 5 μm Spherisorb C18 ODS (2) and operated in an isocratic mode at a mobile phase (0.005 M ammonium dihydrogen phosphate-acetonitrile-methanol; 8:72:20, v/v/v) flow rate of 1.5 ml/min.

Preparation of Standard Solutions

A stock standard solution (1 mg/ml tylosin) was prepared by dissolving 1.053 mg tylosin tartrate in 100.0 ml methanol in a volumetric flask. From this stock solution, working standard solutions with tylosin concentrations of 5, 10, 20, and 40 μg/ml were made by appropriate dilution with methanol.

Extraction Of Tylosin From Tissue

Five g of homogenized tissue were accurately weighed into each of four 50 ml polypropylene tubes. Individual tissue samples were then fortified with 50 μl of 5, 10, 20, and 40 μg/ml tylosin working standard solutions to produce samples with tissue equivalencies of 50, 100, 200, and 400 ng/g, respectively. A 5.0 g tissue sample with no tylosin added served as control. Two hundred μl of 0.05 M $NH_4H_2PO_4$ (pH = 8) were added to each tube, and vortex-mixed. Twenty-five ml of dichloromethane were added to each sample, and the tube was screw-capped and mixed on a shaker (Mechanical, Eberbach flat bed) for 30 minutes. After centrifugation at 3600 g for 10 min, the liquid layer was transferred into a 125 ml separatory funnel, from which the lower dichloromethane layer was drained into a 100 ml beaker.

The remaining upper aqueous layer was returned to the tissue plug in the polypropylene tube and the extraction steps were repeated. The dichloromethane layer from the second extraction was drained into the beaker containing dichloromethane from the first extraction.

Clean Up On Silica Gel Solid Phase Extraction Cartridge

A silica gel cartridge was mounted onto a vacuum manifold. A 75 ml solvent reservoir was mounted onto the silica gel cartridge via an adapter and the cartridge was conditioned with 10 ml petroleum ether followed by 20 ml dichloromethane (Note: It is important not to allow the cartridge to run dry at this stage). The dichloromethane tissue extract was loaded onto the conditioned cartridge and pulled through the cartridge with vacuum at a flow rate of 3 ml/min. The cartridge was washed with 20 ml dichloromethane and air was drawn through the cartridge for 5 min. The adapter and reservoir were removed from the cartridge and tylosin was eluted from the cartridge with 2 ml methanol into a 15 ml glass centrifuge tube. The contents of the tube were evaporated to dryness under a stream of nitrogen in a 45 - 50°C water bath. Two ml of mobile phase were added to the residue in the tube and left to stand in the 45 - 50°C water bath for 10 min and vortex-mixed. The dissolved sample was allowed to cool to room temperature and filtered through a 0.45 μm ACRO LC 13 filter (Gelman Science, Montreal, Quebec, Canada) into LC sample vials for HPLC analysis.

RESULTS AND DISCUSSION

Figure 1A shows a typical UV response from 50 ng tylosin standard injected onto the HPLC column. Figures 1B, 1C, and 1D show chromatograms of extracts from blank beef muscle, and from blank beef muscle fortified with appropriate standards to produce tissue equivalencies of 50 and 400 ng/g tylosin, respectively. Under the HPLC conditions described in this paper, tylosin eluted from the C18 column at a retention time of 9.5 min and was resolved completely from other endogenous components in the muscle, liver, and kidney tissue extracts. In the HPLC methodology previously reported by Moats et al. (6), the retention time of tylosin was claimed to be so dependent on the tissue matrix being analyzed that modification of the composition of the mobile phase was required for each different tissue matrix. In contrast, our mobile phase used for the chromatographic analysis of tylosin in this paper did not result in any shifts in the retention time of tylosin at the optimized column flow rate of 1.5 ml/min, regardless of whether the tissue being analyzed was liver, kidney, or muscle.

The average procedural recovery calculated by comparing the UV response from tylosin obtained after extraction and HPLC analysis with that from an equivalent tylosin external standard was found to be 82 ± 4 % for muscle, 85 ± 4 % for kidney, and 81 ± 3 % for liver (n = 16), respectively. Blank tissue samples (Figure 1B) treated according to the described methodology did not show any peaks eluting at the

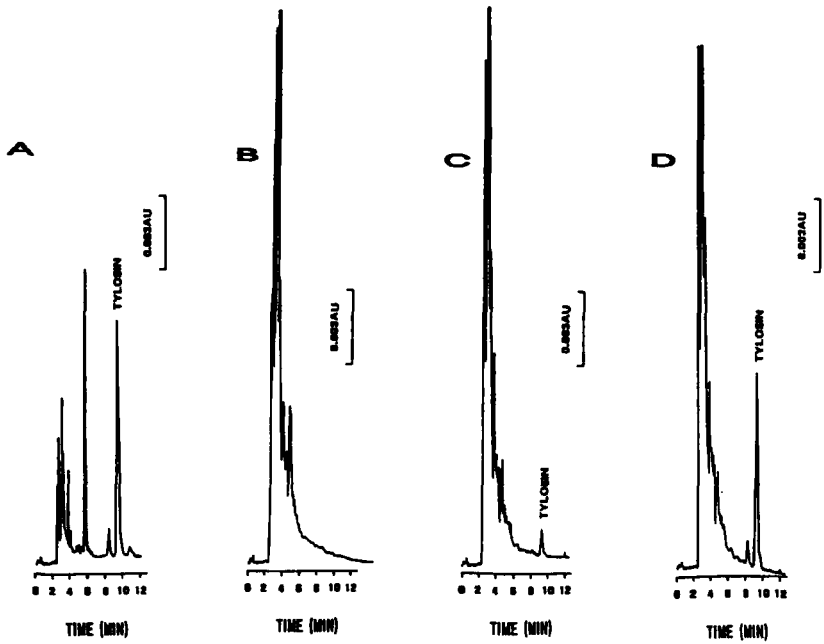

Figure 1. (A) 50 ng tylosin working standard injected onto the column [equivalent to 400 ng/g tissue sample]. Extracts from (B) blank drug-free muscle tissue, (C) blank muscle tissue fortified with tylosin at a concentration of 50 ng/g, and (D) blank muscle tissue fortified with tylosin at a concentration of 400 ng/g.

retention time for tylosin. The macrolide antibiotics erythromycin, oleandomycin, and tilmicosin, and sulphamethazine, which is sometimes incorporated into commercial formulations of tylosin, did not interfere with the chromatographic analysis for tylosin.

The intra-assay precision of the method developed was assessed by replicate analyses (n = 4) of blank, drug-free tissues fortified with various concentrations of tylosin. Average intra-assay variabilities of 4.6, 3.4, and 3.4 % were calculated for bovine muscle,

TABLE 1
Estimation of the Intra-assay Precision for the Analysis of Tylosin in Bovine Tissues

Tissue Matrix	Concn Added (ng/g)	Measured Concn Mean ± S.D.(n=4) (ng/g)	Coefficient of Variation (%)	Accuracy[1] (%)
Muscle	50.0	53.3 ± 3.3	6.2	107
	100.0	98.0 ± 5.5	5.6	98
	200.0	196.9 ± 5.1	2.6	98
	400.0	401.2 ± 16.	4.1	100
Kidney	50.0	42.7 ± 2.2	5.0	85
	100.0	102.8 ± 2.7	2.6	102
	200.0	208.7 ± 8.6	4.1	104
	400.0	396.2 ± 7.2	1.8	99
Liver	50.0	48.8 ± 1.6	3.3	98
	100.0	102.8 ± 4.0	3.9	102
	200.0	197.7 ± 7.8	4.0	99
	400.0	400.8 ±10.5	2.6	100

[1] Accuracy = (Measured Concentration x 100)/Concn added

kidney, and liver, respectively, over the range of concentrations studied (Table 1).

Blank, drug-free tissue samples fortified with tylosin at concentrations ranging from 60 - 320 ng/g were also analyzed on four consecutive days to determine the day-to-day variation (inter-assay precision) of the method developed (Table 2).

This table shows that inter-assay variabilities obtained for bovine muscle, kidney, and liver were less than 10 % and were not significantly different from those obtained from the intra-assay.

Calibration curves obtained from plots of the means of UV response in absorbance units for tylosin (y) versus the concentration (x) [tissue equivalencies in ng/g] added, were linear over the range of concentrations studied and described by the following linear regression equations:

TABLE 2
Estimation of the Inter-assay Precision for the Developed Analytical Method

Tissue Matrix	Concn added (ng/g)	Measured Concentration Day 1	Day 2	Day 3	Day 4	Measured Mean Concn ± S.D.	C.V. (%)
Muscle	60.0	55.6	62.3	53.7	52.0	55.9± 4.5	8.1
	320.0	328.9	326.0	320.0	328.0	325.7± 4.0	1.2
Kidney	70.0	63.9	69.5	70.7	69.3	68.4± 3.0	4.4
	320.0	320.9	333.0	304.0	306.0	316.0±16.6	4.3
Liver	70.0	68.5	70.1	72.1	70.3	70.3± 1.5	2.1
	320.0	342.0	297.0	283.0	317.0	309.8±25.6	8.3

$y = 0.186x - 0.77$ ($r = 0.999$) for beef muscle; $y = 0.179x + 2.26$ ($r = 0.999$) for beef kidney; and $y = 0.162x + 0.9$ ($r = 0.999$) for beef liver. This method has a detection limit of 15 ng/g (S/N = 3).

The HPLC procedure described in this paper is simple, accurate, rapid, reproducible, and sensitive, thus making it suitable for routine use.

ACKNOWLEDGEMENTS

The authors wish to express their sincere gratitute to Drs. George Lee, Syntex Research, and Earl Gardiner, Agriculture Canada, for their valuable suggestions and comments during the preparation of this manuscript.

REFERENCES

1. Takatsuki, K., Ushizawa, I. and Shoji, T., Gas Chromatographic - Mass Spectrometric Determination of Macrolide Antibiotics in Beef and Pork Using Single Ion Monitoring, J. of Chromatogr., 391, 207, 1987.

2. Lauridsen, M. G., Determination and Depletion of Residues of Carbadox, Tylosin, and Virginiamycin in Kidney, Liver, and Muscle of Pigs in Feeding Experiments, J. Assoc. Off. Anal. Chem., 71 (5), 921, 1988.

3. Coleman, M. R. and Mowrey, D. H., Turbidimetric Assay of Tylosin in Animal Feeds Containing Urea, J. Assoc. Off. Anal. Chem., 73 (6), 927, 1990.

4. Food and Drug Regulations - Amendment Schedule No. 753, Canadian Food and Drug Act, Canada Gazette Part I, 3546, Sept. 1990.

5. Moats, W. A., Chromatographic Methods for Determination of Macrolide Antibiotic Residues in Tissues and Milk of Food - Producing Animals, J. Assoc. Off. Anal. Chem., 68 (5), 980, 1985.

6. Moats, W. A., Harris, E. W. and Steele, N. C., Comparison of Liquid Chromatographic and Bioassay Procedures for Determining Depletion of Intramuscularly Injected Tylosin, J. Assoc. Off. Anal. Chem., 68 (3), 413, 1985.

7. Petz, M., Solly, R., Lymburn, M. and Clear, M. H., Thin-Layer Chromatographic Determination of Erythromycin and Other Macrolide Antibiotics in Livestock Products, J. Assoc. Off. Anal. Chem., 70 (4), 691, 1987.