Application Note 00526

Low-Level Detection of Tylosin Using the 320-MS Triple Quadrupole Mass Spectrometer

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Introduction

The presence of antibiotic residues in animals, arising from additives to their feed or water to promote growth or for therapeutic purposes, is widespread. The EU regulations specify ppb tolerance levels in different animals. One class of antibiotics, macrolides, such as erythromycin, spiramycin, and tylosin, are used as feed additives to promote growth in veterinary medicine. The EU tolerance levels are about 100 – 400 ppb (after extraction) depending on the animal tissue tested.[1] Tylosin was investigated in the current work. The 320-MS Triple Quadrupole Mass Spectrometer can accurately detect and quantitate tylosin over a broad concentration range and below EU required tolerance limits.

Instrumentation

- Varian 212-LC Binary Gradient LC/MS Chromatography Pump (2)
- Varian 320-MS Triple Quadrupole Mass Spectrometer equipped with an ESI source
- Varian ProStar™ 430 Autosampler

Materials and Reagents

All solvents (reagent or HPLC Grade) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Tylosin Tartrate (Part # 93806-1G) was purchased from Sigma- Aldrich Corporation (St. Louis, MO).

Sample Preparation

Stock solutions were purchased at a concentration of 1 mg/mL in methanol. Further dilutions of the stock solution were carried out in a 1:1 mixture of Water:Methanol.

Sample Preparations:

Solutions ranging in concentration from 0.5-500 pg/µL from the stock solution were prepared for calibration curve purposes. The samples were run in triplicate.

Instrument Conditions

LC Conditions:

Column: Polaris C18-A 5µ 100 X 020 (Varian Part# A2000100X020)

Solvent A: Water

Solvent B: 0.1% formic acid in acetonitrile

Flow rate: 0.2 mL per min

Injection Volume: 5 µL

LC Program:

Time (min)	% B
0	5
5:00	95
6:30	95
8:00	5
10:00	5

Mass Spectrometry Conditions:

Ionization Mode: ESI positive

Nebulizing Gas Pressure: 55 psi

Drying Gas Pressure: 36 psi at 400 °C

Dwell Time: 0.400 s for each transition

MS/MS Conditions:

	Tylosin
Precursor Ion (m/z)	916.5
Capillary (V)	160
Product Ions (m/z)	174 772
Collision Energy (V)	32 (m/z 174) 27 (m/z 772)

The calibration curve was calculated using the 916.5 ➔ 174 transition.

Discussion

Figure 1 shows the calibration curve for triplicate injections the concentration range of 0.5 – 500 pg/µL. The R^2 value = 0.998 over the concentration range.

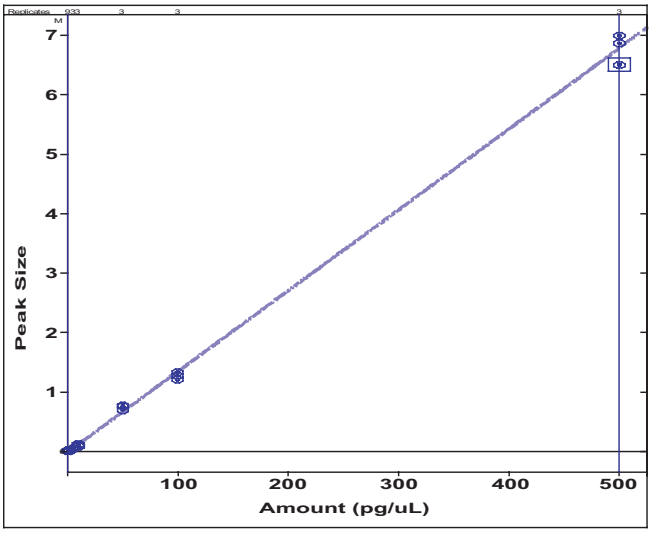

Figure 1 Calibration Curve of Tylosin (916.5➔ 174) over the range of 0.5 – 500 pg/µL.

The SRM chromatogram of 0.5 pg/µL of tylosin is shown in Figure 2. A concentration of 0.5 pg/µL is the limit of quantitation (LOQ with a S:N ~ 300:1) and the limit of detection (LOD) is 0.2 pg/µL (LOD with a S:N ~ 30:1). These values are well below the required tolerance limits for this particular antibiotic.

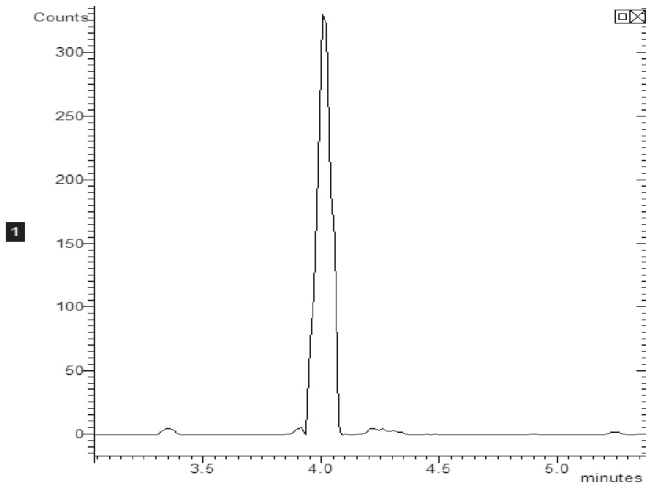

Figure 2 SRM Chromatogram of the 916.5➔ 174 m/z transition of Tylosin at a concentration of 0.5 pg/µL.

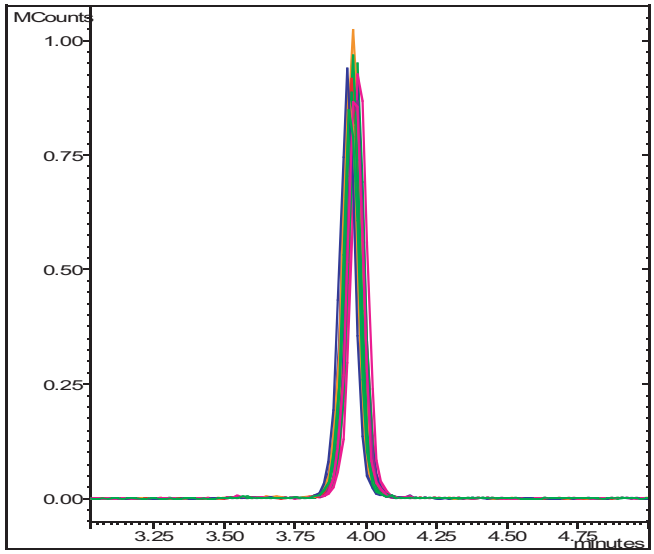

Figure 3 Overlaid SRM Chromatograms of 20 consecutive Tylosin (916.5➔ 174) injections.

Both retention time and area reproducibility are extremely important in determining how well an instrument can perform on a day-to-day basis. Figure 3 shows the SRM chromatograms of 20 of consecutive injections of Tylosin at a concentration of 10 pg/µl.

The % RSD (Area Reproducibility) and % RSD (Retention Time) for 20 injections were 6.14 and 0.25, respectively. These RSD values are the direct result of the complete Varian System used in the current analysis.

Conclusion

The measurements performed show that good quantitation and reproducibility can be obtained for a particular class of antibiotics. The detection limit of tylosin using the 320-MS Triple Quadrupole Mass Spectrometer falls well below the tolerance limits set forth by the EU with good reproducibility.

[1] Gentili, A., Perret, D., and Marchese, S., Trends in Analytical Chemistry, **24** (7) 704.

These data represent typical results.
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SI-0526