Application Note 00542

Detection of Lasalocid in Animal Feed Extract using the Varian 320-MS Triple Quadrupole Mass Spectrometer

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Introduction

Lasalocid belongs to a group of antibiotics known as ionophores, which are derived from a *Streptomyces* species. High doses of these drugs, whether administered to poultry or through high concentrations in animal feed, can be highly toxic, and questions are being raised about the potential hazard to humans.[1] This drug is also used in the prophylaxis and treatment of coccidiosis and leukocytozoonosis in poultry. Concern has been raised over the misuse of these drugs at harmful or lethal levels when these drugs are used in animal feed. In Norway, for example, the concentration of lasalocid added to chicken feed varies between 75 to 125 mg/kg.[2]

The focus of this study was to examine the ability of the Varian 320-MS Triple Quadrupole Mass Spectrometer to detect lasalocid in animal feed.

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). Lasalocid (both a standard solution and matrix sample) were provided by a Dept of Agriculture Laboratory. At the time that the work was being performed, a standard of this compound was not readily available.

Sample Preparation

The standard solution was at a concentration of 864 ng/µL. Further dilutions of the standard solution were carried out in a 1:1 mixture of Water:Methanol.

Sample Preparations:

Solutions ranging in concentration from 0.4 – 40 pg/µL from the standard solution were prepared for calibration curve purposes. The samples were run on a 320-MS Triple Quadrupole Mass Spectrometer.

Instrument Conditions

LC Conditions:

Column: Pursuit XRs-DP 3µ DP 150 mm x 2 mm (Varian Part# A6021050X020)

Solvent A: Water

Solvent B: 0.1% Formic Acid in 1:1 (v/v) Acetonitrile:Methanol

Flow rate: 0.2 mL per min

Injection Volume: 5 µL

LC Program:

Time (min)	% B
0	5
1:00	95
4:00	95
5:00	5
7:00	5

Mass Spectrometry Conditions:

Ionization Mode: ESI positive/ESI Negative

Nebulizing Gas Pressure: 55 psi

Drying Gas Pressure: 38 psi at 350 °C

Dwell Time: 0.333 s for each transition

MS/MS Conditions:

	lasalocid -	lasalocid +
Precursor Ion (m/z)	589.4	613.3
Capillary (V)	-155	100
Product Ions (m/z)	235 173	377
Collision Energy (V)	34 45	34.5

The calibration curve was calculated using the 589.4 ➔ 235 transition, with 589.4 ➔ 173.0 and 613.3 ➔ 377 used as qualifier ions.

Discussion

Figure 1 shows the SRM chromatograms of all three transitions that were used in the current analysis for the lasalocid standard solution at a concentration of 2 pg/µL.

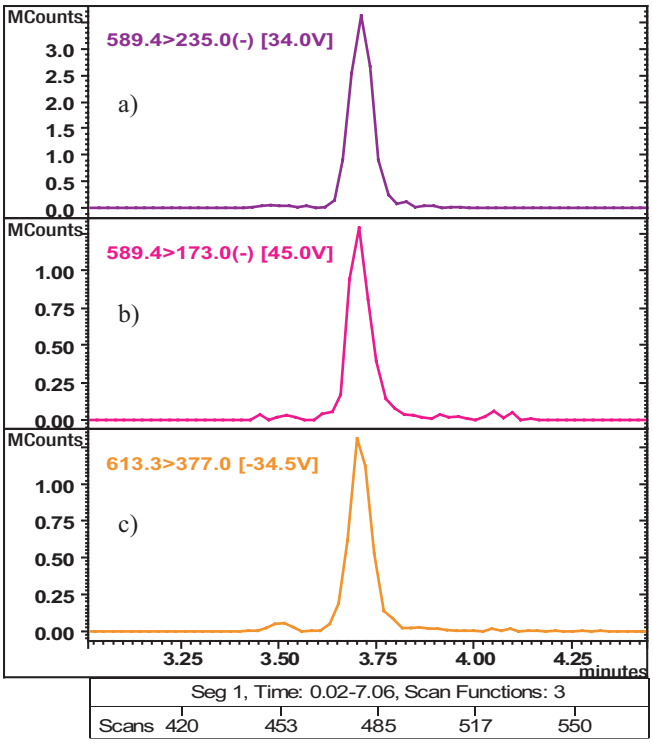

Figure 1 SRM Chromatograms of Lasalocid transitions: a) 589.4 ➔ 235, b) 589.4 ➔ 173, and c) 613.3 ➔ 377 at a concentration of 2 pg/µL.

The calibration curve over the concentration range of 0.4 – 40 pg/µL is shown in Figure 2.

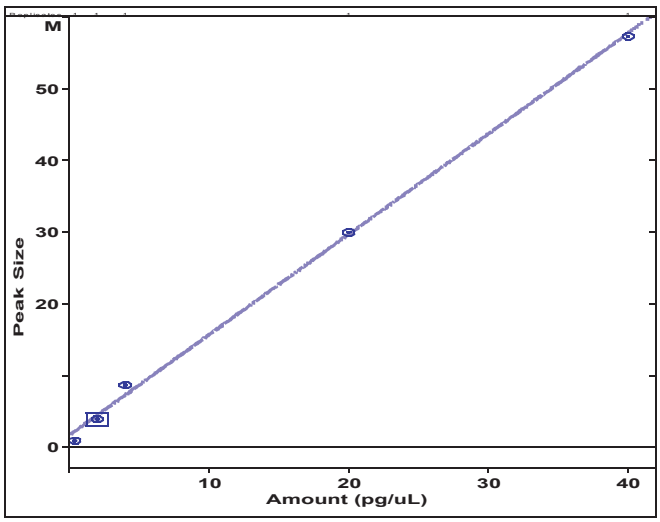

Figure 2 Calibration Curve of Lasalocisd (589.4 ➔ 235) over the concentration range of 0.4 – 40.0 pg/µL.

The animal feed sample that was provided to Varian was at a concentration of 29.5 mg/kg. The samples we received were extracted in 0.1 % HCl in Methanol and the final concentration was 3.3 ng/µL. This sample was diluted to a concentration of 33 pg/µL in a 50:50 CH_3OH/H_2O mix. Figure 3 shows the SRM chromatogram of lasalocid in animal feed extract for the transition of 589.4 ➔ 235.

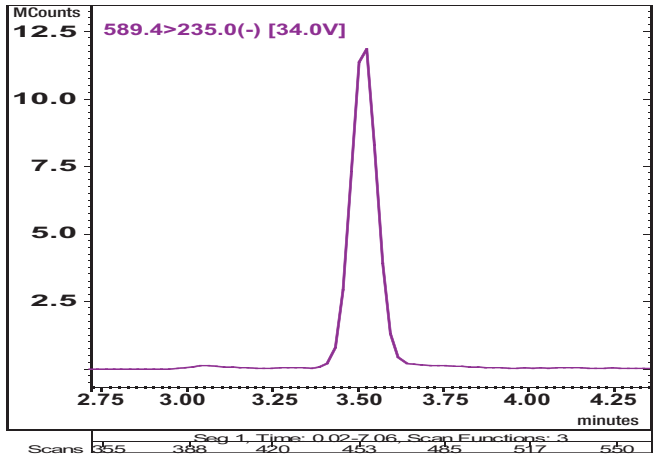

Figure 3 SRM Chromatogram of Lasalocid (589.4 ➔ 235) in animal feed extract.

The overall sensitivity of the instrument to detect lasalocid in animal feed extract remains above those levels required by the EU. Based on the S/N (1500 (peak to peak)) of this matrix sample, the Varian 320-MS Triple Quadrupole MS could sensitively detect lasalocid in animal feed extracts at much lower levels (µg/kg).

The Area Reproducibility was measured over the course of 20 injections for all three transitions. The % RSD = 6.06, 5.50, and 5.79 for the 589.4 ➔ 235, 589.4 ➔ 173, and 613.3 ➔ 377, respectively. The plot of these areas over the 20 injections is shown in Figure 4.

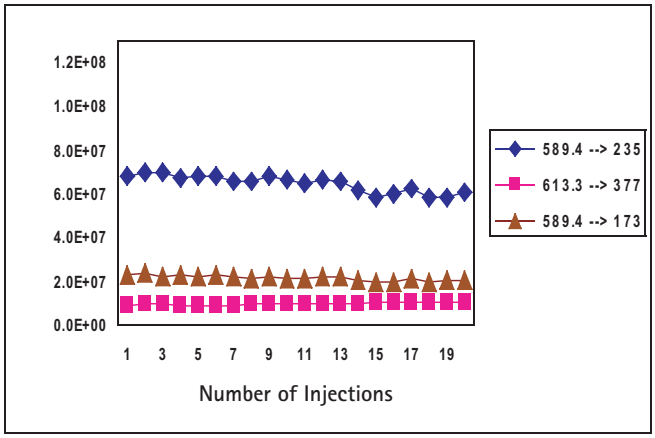

Figure 4 Plot of Area versus Number of Injections for all three transitions of Lasalocid in animal feed extract.

The addition of the animal feed extract sample to the LC/MS system did not affect either the reproducibility or sensitivity of instrument. In addition, the fast positive/negative switching capabilities of the 320-MS instrument are highlighted since accurate reproducibility and quantitation were achieved using either the positive or negative transitions. The Varian XRs-DP column provided reproducible peak shape and consistent retention times.

Conclusion

The detection of lasalocid in animal feed has been demonstrated with good reproducibility with MS/MS transitions in both positive and negative mode. The signal response of lasalocid in animal feed shows that the concentration could be decreased significantly and still be detected.

[1] Mortier, L., Daeseleire, E., and Van Peteghem, C., Rapid Commun. Mass Spectrom. 2005; 19: 533-539.

[2] Hormazabal, V., Yndestad, M., and Ostensvik, O., J. Liq. Chrom. and Related Tech., 2002: 25 (17); 2655 – 2663.

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