Comparison of Florfenicol Depletion in Dairy Goat Milk Using Ultra-Performance Liquid Chromatography with Tandem Mass Spectrometry and a Commercial On-farm Test

Emily D. Richards, PharmD1,2, Richard V. Pereira, BVSc, PhD3\*, Jennifer L. Davis, DVM, PhD1,4, Joan D. Rowe, DVM, PhD2, Maaike O. Clapham1,2, Scott E. Wetzlich1,2, Benjamin A. Rupchis5, Lisa A. Tell, DVM1,2\*

1Food Animal Residue Avoidance and Depletion Program, USA

2Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA

3Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA

4Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA 24061, USA

5Department of Animal Science, University of California-Davis, Davis, CA 95616, USA

**\*Co-Corresponding Authors:** Richard V. Pereira, and Lisa A. Tell, 1 Garrod Dr, Davis, CA, rvpereira@ucdavis.edu; latell@ucdavis.edu

Keywords: florfenicol, goat, extra-label drug use, drug residue, milk.

Supplementary Material

# Supplementary Tables

**Supplemental Table 1.** Ultra-performance liquid chromatography (UPLC) gradient method for the mobile phase used for the florfenicol analysis in goat milk samples following administration of florfenicol 40 mg/kg subcutaneously twice 4 days apart in lactating does.

|  |  |  |
| --- | --- | --- |
| **Time**  **(min)** | **%A**  **(10mM NH4Ac+0.05% HAc)** | **%B**  **(ACN)** |
| 0.00 | 87 | 13 |
| 0.25 | 87 | 13 |
| 0.75 | 2 | 98 |
| 1.25 | 2 | 98 |
| 1.26 | 87 | 13 |
| 3.25 | 87 | 13 |

%A = mobile phase A, 0 mM ammonium acetate (NH4Ac) + 0.05% (v/v) acetic acid (HAc) in H2O: %B = mobile phase B, 100% ACN.

**Supplemental Table 2.** Multiple reaction monitoring (MRM) transitions and specific mass spectrometry tuning parameters for the quantification of florfenicol and florfenicol amine in goat milk samples following administration of florfenicol 40 mg/kg subcutaneously twice 4 days apart in lactating does.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Analyte** | **Parent Ion**  **(amu)** | **Product Ion**  **(amu)** | **Cone Energy**  **(V)** | **Collision Energy (eV)** | **Quant/Qual Transition** |
| Florfenicol  amine | 248.1  [M+H]+ | 130.3 | 24 | 21 | Quantifier |
| 248.1  [M+H]+ | 230.0 | 24 | 12 | Qualifier 1 |
| Florfenicol  amine-d3  (IS) | 251.1  [M+H]+ | 233.1 | 24 | 12 | Quantifier |
| 251.1  [M+H]+ | 130.5 | 24 | 22 | Qualifier 1 |
| Florfenicol | 356.0  [M-H]- | 185.0 | 26 | 20 | Quantifier |
| 356.0  [M-H]- | 336.0 | 26 | 10 | Qualifier 1 |
| Florfenicol-d3  (IS) | 359.0  [M-H]- | 188.0 | 28 | 20 | Quantifier |
| 359.0  [M-H]- | 339.0 | 28 | 8 | Qualifier 1 |

**Supplemental Table 3.** Mass spectrometer tuning parameters for the detection of florfenicol and florfenicol amine in goat milk samples following administration of florfenicol 40 mg/kg subcutaneously twice 4 days apart in lactating does.

|  |  |
| --- | --- |
| **Parameter** | **Value** |
| Capillary (kV) | 0.50 |
| Cone (V) | 25 |
| RF (V) | 2.50 |
| Extractor (V) | 3.00 |
| Source Temperature (°C) | 150 |
| Desolvation Temperature (°C) | 600 |
| Cone Gas Flow (L/h) | 10 |
| Desolvation Gas Flow (L/h) | 1000 |

**Supplemental Table 4.** Physical examination characteristics for five dairy goat does enrolled in a florfenicol milk depletion study following administration of florfenicol 40 mg/kg subcutaneously twice 4 days apart.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Doe | 1 | 2 | 3 | 4 | 5 |
| Breed | Alpine | Saanen | Saanen | LaMancha | Alpine x LaMancha |
| Age (yr) | 3 | 5 | 2 | 2 | 2 |
| Weight (kg) | 90 | 111.5 | 113 | 77.5 | 80 |
| BCS | 3.25 | 3.75 | 3.75 | 3 | 3.25 |
| FAMACHA® Score | 2 | 2 | 1 | 2 | 1 |
| Lactation Number | 2 | 4 | 1 | 1 | 1 |
| Days in Milk1 | 5 | 11 | 5 | 11 | 4 |
| Milk Production2 (lbs) | 7.59 ± 1.43 | 6.64 ± 1.19 | 7.59 ± 1.34 | 5.27 ± 0.84 | 5.08 ± 0.97 |

1. Days in milk for first dose of florfenicol

2. Average ± standard deviation milk production in lbs for one milking. The farm milked does twice a day.

BCS= body condition score, on a scale of 1 to 5; FAMACHA® = Faffa Malan Chart.