Supplemental Digital Content

Methods

Study Design

The animal studies were performed according to NIH guidelines in AAALC accredited facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The protocols were approved by Institutional Animal Care and Use Committees at The University of Texas Medical Branch (UTMB) in Galveston, TX and at the Biological Test Center (BTC) in Irvine, CA. Animals are not routinely in pain for dosing or sample collections for this type of pharmacokinetic study. Prior to vaginal ring insertion and prior to euthanasia, each sheep was anesthetized with ketamine IM (10 mg kg^{-1}) followed by ketamine IV (10 mg kg^{-1}), and 1-2% isoflourane in 50:50 air:oxygen gas mix with ventilatory support (UTMB) or by ketamine hydrochloride (50 mg mL^{-1}) and diazepam (2.5 mg mL^{-1}) *via* IV at a dose volume of 0.7 to 1 mL per 10 kg body weight (BTC). Upon completion of the study euthanasia was carried out by saturated potassium chloride given under general anesthesia by IC injection (UTMB) or by IV of commercial euthanasia solution according to SOP 1001307 (BTC).

Samples collection, processing, and analysis

Samples were prepared for bioanalysis by filtration (CVL) or protein precipitation (plasma, pulverized tissue) and were analyzed by LC/MS (Agilent Series 1100 system) with lamivudine as the internal standard under the following conditions: Poroshell SB-C3 Rapid column (2.1 × 150mm 5 μm, Agilent); gradient program, 3 min hold 100: 0 A:B (A, 0.1% acetic acid in water; B, 0.1% acetic acid in acetonitrile), 5 min ramp to 0:100 A:B; 2 min hold at 0:100.