The importance of food safety through the reduction of residues in our food supply cannot be overemphasized. Food safety remains a major challenge confronting contemporary society. Analytical methods are needed to generate the data on which dietary exposure assessments are based and to enforce statutory maximum residue limits (MRLs) that are set. Diminazene aceturate is one of the few drugs used for animal trypanosomosis. Because of its wide use in livestock, the risk of unwanted residues in edible products may exist. A competitive enzyme-linked immunosorbent assay (ELISA) for determination of diminazene residues in edible animal tissues after extraction in 0.1 M borax at pH 9.7 was investigated. The assay used rabbit anti-diminazene polyclonal antibody on the solid phase support. Horseradish peroxidase-labeled diminazene was incubated with sample overnight at 4°C. After five washes with buffer enzyme activity was determined by adding tetramethyl-benzidine and hydrogen peroxide as substrate. The resulting blue colour whose intensity was inversely proportional to the drug concentration changed to yellow when the reaction was stopped by addition of 0.1 M orthophosphoric acid.

The assay was optimized and validated for determination of diminazene in tissues. The assay exhibited high specificity (99.997%) for diminazene recognizing only isometamidium at 0.003% and this may be contributed by the amidinophenyl that is common in both drugs. Recoveries from spiked tissues were above 77% while Dilutional parallelism experiments demonstrated a recovery of 96.0% ± 9.5%. The limit of detection (LOD) for the assay was 2.4 ng/g for muscle, 2.5 ng/g for liver and 2.2 ng/g for kidney while limits of quantification (LOQ) were 5.51 ng/g, 4.11 ng/g and 3.74 ng/g respectively. The LODs are 4.4x10^3 to 3.5x10^3 lower than the MRLs that are 500 μg/kg, 12,000 μg/kg and 6,000 μg/kg of muscle liver and kidney respectively. Assay precision was characterized by a within assay coefficient of variation (CV) of 2.4% and between assays CV of 15.5%. When diminazene was administered intramuscularly at 3.5 mg/kg to five goats that were sacrificed seven days later, the mean diminazene residue levels were 0.75 μg/g ± 0.14 μg/g for skeletal muscle, 32.05 μg/g ± 5.7 μg/g for liver and 4.29 μg/g ± 0.66 μg/g for kidney. The analysis of tissue samples collected from slaughterhouses around Nairobi showed that out of 35 muscle samples, only one was positive and had a diminazene concentration of 0.039 μg/g. Four out of 32 kidney samples were positive for diminazene with levels of 0.63, 1.66, 2.61 and 3.96 μg/g. From ten liver samples two were positive with levels of 1.07 and 1.74 μg/g. From this analysis none of the positive samples had levels above the MRL values. This study has demonstrated that competitive ELISA can be employed for the determination of diminazene residues.

The results of this study are relevant to food scientists, toxicologists and analysts working in the area of detection and safety assessment of food residues, companies developing veterinary drugs, regulatory bodies involved in safety assessment of veterinary drugs and residue monitoring and to regulatory bodies responsible for veterinary drugs registration.