**Introduction**

Nitrofurans have been used in veterinary practice as antibacterial agents and their use in food producing animals is prohibited in many countries. Nitrofurantoin, furazolidone, furaltadone and nitrofurazone are the four main nitrofuran antibiotics. Studies have shown them to be rapidly metabolised in vivo; however, the tissue bound metabolites, AHD, AMOZ, AOZ and SEM can persist for at least 6 weeks. The development of convenient methods enabling their detection is of interest for monitoring and regulatory applications.

**Materials and Methods**

**Tissue Sample Preparation**
- Addition of K2HPO4 and NaOH
- Vortexing, centrifugation and decantation
- Resuspension in diluent/wash buffer and vortexing
- Combination of supernatants and filtration
- Vortexing, centrifugation and decantation
- Vortexing and incubation (2h, 50°C)

**Analytical parameters**
- Limit of Detection (LOD)
- Specificity/Cross-reactivity

**Results**

**Conclusion**

Data indicate that the developed competitive ELISAs detect 4 nitrofuran metabolites with LODs ranging from 0.2 ng/g (AMOZ) to 0.6 ng/g (SEM) in tissue samples. Overall typical recovery was >70%. Intra-precision values were typically %CV<9% over a range of concentration levels.

Use of multi-analyte calibrators and multi-analyte conjugate simplifies the assay procedure, avoids the use of different calibrators and conjugates for the analysis of 4 nitrofuran metabolites from 1 sample preparation.

After sample preparation, for each assay 40 samples can be screened in 90min.

These assays represent a valuable and convenient analytical tool for the in vivo determination of nitrofuran metabolites in tissue samples.