Phenylbutazone was one of the first non-steroidal anti-inflammatory drugs (NSAIDs) approved for use in horses and dogs. High doses of phenylbutazone may be considered a rules violation under some equestrian organizations, as the drug may remain in the bloodstream four to five days after administration. Currently, the use of phenylbutazone in food producing animals is prohibited in most countries. MRLs have not been set for phenylbutazone residues and any detection is considered a violation. We report the development of a competitive ELISA for the detection of phenylbutazone and its metabolite o xo phenbutazone in serum/plasma samples, which is of interest for monitoring and regulatory purposes.

### Methodology

**Competitive ELISA**

In-house made capture antibody was immobilised and stabilised on 96 well microtitre plates. The assay is based on a competitive reaction where any free analyte contained in the standards/samples competes for binding sites of the capture antibody with homosalid

#### Limit of Detection (LOD)

The LOD was defined as mean concentration of negative samples + 3SD. LOD was 2.01 ng/ml for phenylbutazone. Intra precision values are typically %CV were assessed. Results were expressed as % recovery.

#### Results

The analytical performance of the developed ELISA kit (PB3456, Randox Laboratories, Crumlin, UK) was assessed. Results were expressed as % recovery.

#### Conclusion

- Data indicate that the developed competitive ELISA detects phenylbutazone and its metabolite o xo phenbutazone in serum/plasma samples.
- Simple sample preparation, only dilution required (1/5).
- LOD = 2.01 ng/ml for phenylbutazone. Intra precision values are typically %CV < 3% over a range of concentration levels and the % recovery was 92% for a concentration 5 ng/ml.
- Once applied to the microtitre plate 40 samples can be screened in 90 min.
- The developed ELISA represents a valuable and convenient analytical tool that can be used for the in vitro determination of phenylbutazone and o xo phenbutazone in serum/plasma samples.

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**Chemical Structures**

![Phenylbutazone](image)

![Oxyphenbutazone](image)