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## Validation of a monoclonal antibody-based ELISA for the quantification of the furazolidone metabolite (AOZ) in eggs using various sample preparation

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A monoclonal-based ELISA, coupled with an assay buffer, solvent and solid phase extraction procedures, was validated for use in the monitoring of egg samples for 3-amino-2-oxazolidinone (AOZ). The procedures allow the detection of protein bound AOZ in the form of 2-nitrophenyl derivative (NPAOZ) in sample supernatant or extract after acid hydrolysis and derivatisation with o-nitrobenzaldehyde. The assays were validated according to criteria set down by Commission Decision (2003) for the performance and validation of analytical methods for chemical residues. The detection capability of ELISA's for AOZ in eggs (set on the basis of acceptance of no false negatives) was 0.6, 0.3 and 0.3 µg/kg for buffer, solvent and solid phase extraction, respectively. These values are well below the maximum required performance limit (MRP) of 1 µg/kg for tissue bound residues of nitrofuran antibiotics. An excellent correlation of results ( $r = 0.99$ ,  $n = 14$ ) obtained by the ELISA and LC-MS/MS techniques within the concentration range of 0–5 µg/kg was found in the incurred egg samples. The eggs collected from layer chickens fed 30 and 400 mg/kg of furazolidone for 10 days were monitored by ELISA until AOZ concentrations approached the LoD.

**Keywords:**

monoclonal ELISA; nitrofuran; AOZ; eggs; sample preparation; validation

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