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 (MRLP) 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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