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Validation of radioimmunoassay screening methods for β -agonists in bovine liver according to Commission Decision 2002/657/EC

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Validation of radioimmunoassay screening methods for β -agonists in bovine liver according to Commission Decision 2002/657/EC

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Validation studies were carried out on a multi-residue screening method for anilinic type β -agonists (clenbuterol, mabuterol, brombuterol, cimaterol, cimbuterol, mapenterol, clenpenterol) and a method for the phenolic type β -agonist, salbutamol, in bovine liver. The validation was performed according to the European Union Commission Decision 2002/657/EC (European Commission 2002), which establishes criteria and procedures for the determination of parameters such as the detection capability ($CC\beta$), specificity, stability of standard solutions and stability of the analyte in matrix. $CC\beta$ values for the eight target compounds were between 0.25 and $0.5 \mu\text{g kg}^{-1}$. The stability of standard solutions and analytes in matrix and the specificity of the antibody were characterized. The methods are applicable for qualitative screening of β -agonists for regulatory programmes according to European Union performance requirements, or as a semi-quantitative research tool for known target compounds.

Keywords: β -agonists; radioimmunoassay; residues; validation

Introduction

β -Agonists are frequently used in farm animals for the treatment of pulmonary diseases and bronchospasm. Lipolysis and increased protein synthesis are well-known side-effects and, therefore, these substances can also be misused as growth promoters (Buttery and Dawson 1990). However, because of the potential harmful effects on human health of residues of these drugs in food, the European Union has banned their use in animal production systems through Council Directive 96/22/EC (European Commission 1996).

Clenbuterol and mabuterol are sympathomimetic bronchodilating agents belonging to the family of β_2 -agonists. These compounds are used in veterinary and human medicine for the treatment of chronic and obstructive pulmonary diseases (Murai et al. 1984; Nazzari 1985). Clenbuterol is probably the most well known of the aniline type β_2 -agonists, and is the most lipophilic. When administered orally in livestock, it can be absorbed by liver, lungs, kidneys and secretor organs, such as pancreas and supra-renal glands (Miller et al. 1988; Sauer et al. 1995; Smith and Paulson 1997; The European Agency for the Evaluation of Medical Products (EMEA) 2000). Tissue concentrations of clenbuterol decrease rapidly, with liver, eyeball (retina), hair and feathers showing

the slowest depletion of residues. These organs are, therefore, suitable for detection of the drug during or after the withdrawal periods in the animal (Malucelli et al. 1994; Sauer and Anderson 1994). On the other hand, the metabolism and pharmacokinetic characteristics of mabuterol are largely unknown in livestock, but reliable data from rodent and human studies showed that mabuterol had a long-lasting effect after oral administration and was extensively absorbed along the entire small intestine of rats (Guentert et al. 1984; Yuge et al. 1984). Both clenbuterol and mabuterol have been abused to improve by producers carcass quality and other members of the β -agonist group of drugs may be used in the same way.

Because of the diversity of the substances illegally used in meat production, the presence of these compounds at trace levels and the complexity of the biological matrices analysed, there is a continuous need for development of a multiresidue strategy of analysis, involving a quick and easy sample pretreatment, followed by a specific and sensitive determination of several residues within the same run. A number of techniques have been developed or applied to screen for β -agonists. A surface plasma resonance (SPR) biosensor assay was described by Traynor et al. (2003). The assay was able to detect mabuterol down to