APPLICATION OF STANDARDIZED APPROACHES FOR THE DETERMINATION OF WITHDRAWAL PERIODS OF VETERINARY MEDICINAL PRODUCTSFROM THE ORGANISM OF PRODUCTIVE ANIMALS

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The manuscript summarizes the experience gained by National Reference Laboratory of Control of Veterinary Drugs and Feed Additives Residues of SCIVP in the application of immune enzyme method (ELISA) for studying the levels of active substances residues in the tissues of productive farm animals to safe levels established by the legislation of Ukraine and European Union. The stages of clinical studies of veterinary antimicrobial drugs containing antibiotics of the quinolones group have been considered, that illustrate the application of standardized approaches to assess the suitability of the above-mentioned methods for the purposes of the experiment and deal with the critical points of validation process in accordance with the criteria established by European Commission Decision 657/2002/EC and Reference EU Laboratories in the field of residual quantities control 20/1/2010. The peculiarities of the method are discussed; and the possibilities of their influence on the obtained results and statistical approaches during calculations of predicted periods of productive animals keeping are analyzed in accordance with the recommendations of EMEA/CVMP/036/95-FINAL.

Quinolines of the second generation are most frequently used in veterinary medicine, and enrofloxacin, in particular, which was suggested for the treatment of bovine animals, pigs, dogs, cats, chickens and turkeys in the cases of diseases of respiratory, urinary and digestive systems. For a long time, the use of the above-mentioned drugs is increasingly followed with the cases of lowering their efficacy, which, in turn, leads to the increase of therapeutic doses of the produced drugs and may lead to the exceeding of established regulatory maximum residues limits of enrofloxacin and its metabolites in animal raw materials. These residues can carry significant risks to human health, stimulating the development of bacterial resistance and disturbing the microflora of the intestinal tract.

To reduce the risks to human health, European Union legislation sets the maximum residues levels (MRLs) for enrofloxacin and its metabolite ciprofloxacin in

food products, viz. for muscle tissues of all animals MRL is 100 μg/kg, for liver - 200 μg/kg (chickens, pigs, rabbits) and 300 μg/kg (cattle, sheep, goats); and for milk it is 100 μg/kg (Commission Regulation 37/2009). For the same purpose, the concept of a withdrawal period of residues of active pharmaceutical ingredients from an animal after the completion of their treatment until the concentration of the drug and/or its metabolites in target animal tissues is reduced to a safe level below the established MRL.

The purpose of this publication is to show the main stages of the procedure for determining the withdrawal period for veterinary preparations based on enrofloxacin from tissues (muscle, liver) of broiler chickens and milk of cows. We used RIDASCREEN Chinolone / Quinolones (Art No. R3113) test kits manufactured by R-Biopharm (Germany). Validation of the analytical methodology was carried out according to the EU Decision 657/2002 and Recommendation 20/1/2010, while its basic parameters were set, viz. the percentage of extraction (R, %), the decision limit (technical threshold, CCα), the detection capability (CCβ) for the target samples. The results obtained in the kinetic experiment were counted according to the calculated percentage of extraction: 90.8, 105.6, 102.6% for samples of muscle, liver and milk. The statistical processing of the data obtained was carried out in accordance with the recommendations of EMEA/CVMP/036/95 and EMEA/CVMP/473/98, predicted time of the target animals' period of withdrawal was calculated using WT 1.4 software (for tissue samples) and WTM 1.4 (for milk samples) which involves the use of various statistical tests of the objective assessment of the unification of the data variance.

Based on the experimental data and the results of their statistical processing, the withdrawal period for chicken broilers was calculated to provide the MRL for enrofloxacin residues in muscle tissues at 6.74 days and 6.92 days for liver samples, according to the guidelines, the rounded value is 7.0 days. According to the results of the study, the withdrawal period of enrofloxacin from milk samples of cows was calculated to be 4.47 days, which is 5.0 days. Compliance with these established withdrawals periods provides 95% the probability that enrofloxacin residues level in muscle tissues and liver of chickens and in milk of cows will not exceed the MRL values, taking into account the limits of uncertainty of its methods of control.

However, the results presented in this article considering the analysis of withdrawal periods from target tissues of broiler chickens and milk for individual cows cannot serve as a dogma for all veterinary drugs of this type on the basis of enrofloxacin.

Keywords: WITHDRAWAL PERIOD, METHOD VALIDATION, STATISTICAL ASSESSMENT OF THE RESULTS, ENROFLOXACIN, MRL.