IVERMECTIN

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ADDENDUM

To the monograph prepared by the 54th meeting of the Committee and published in FAO Food and Nutrition Papers 41/13

INTRODUCTION

Background

Ivermectin is widely used as a broad-spectrum antiparasitic drug against nematodes and arthropods in food-producing animals. In human medicine, it is used mainly for the treatment of onchocerciasis.

Ivermectin was previously considered at the 36th, 40th, 44th and 54th meetings of the Committee. At the 40th meeting, the Committee recommended an ADI of 0-1 µg/kg of body weight and MRLs of 100 µg/kg in liver and 40 µg/kg in fat, as ivermectin B_{1a}. At the 54th meeting, the Committee evaluated milk residue data following topical application of the drug to dairy cattle and recommended a temporary MRL of 10 µg/kg for whole milk, also expressed as ivermectin B_{1a}.

The Committee noted that data concerning the limits of detection and limits of quantification for the assay had not been provided and requested that the data for method performance be provided for evaluation in 2002. Additionally, the Committee requested information on routes of administration other than the topical route of administration.

For evaluation at the 58th meeting, information was provided on analytical method performance. Although not requested, a new residue study also was submitted. Alternative routes of administration were not addressed.

RESIDUES IN MILK AND THEIR DETERMINATION

Milk residue depletion study

A milk residue depletion study compliant with Good Laboratory Practices was conducted in cattle (Chick, 2000).

Eight lactating Holstein dairy cows received a single topical administration of ivermectin pour-on at a dose of 0.58 mg active ingredient per kg body weight. One animal served as an untreated control. Triplicate whole milk samples were collected from all animals prior to treatment (day 0) and at approximately 12-hour intervals for days 1 to 9 following treatment. Milk samples were collected from all animals only on the morning of day 10. Samples were stored in a portable freezer at the dairy following collection and subsequently shipped on dry ice to the analytical laboratory.

For milk samples collected on day 1 to day 4, the ivermectin concentrations in both morning and evening milk samples were provided. For samples collected on day 5 to day 10, only the ivermectin concentrations in morning milk samples were reported. The ivermectin concentrations in the samples collected prior to the first administration (all animals) and from the control animal (all samples) were below the Limit of Detection (LOD, <0.02 ng/mL) for the assay. Ivermectin concentrations in milk rose following treatment, reaching peak concentrations 3 to 4 days post-treatment. In two cows, milk residues did not exceed the temporary MRL of 10 µg/kg in any of the analyzed milk samples. For the remaining six animals, peak residues of ivermectin ranged from 14.3 ng/mL to 32.5 ng/mL, determined in milk samples obtained from Day 2 afternoon to Day 4 afternoon milkings. Ivermectin concentrations in the milk declined over the final five days of the study. The incurred milk residues of ivermectin resulting from the administration of a 5 mg/mL pour-on formulation per label directions were less than 50 ng/mL in each of the 120 milk samples collected between 1 and 10 days following treatment. Although all of the milk samples collected from the treated animals contained detectable residues for the entire study period, none of the samples collected on day 9 and day 10 exceeded the temporary MRL, 10µg/kg.

Summary of analytical methods

Sampling

In the field trial conducted in dairy cattle (Chick, 2000), samples were collected in triplicate and immediately placed on ice. Milk samples were shipped to the analytical laboratory on dry ice and were stored at -16 °C pending analysis.

HPLC Assay

An analytical method was submitted for evaluation. The method involved separation by high performance liquid chromatography (HPLC) and detection of derivatised compounds (parent ivermectin, 22,23-dihydroavermectin B_{1a}, and the internal standard, avermectin B_{1a}) by fluorescence.

According to the method (Chick, 2000), milk samples were brought to room temperature and the internal standard was added. Following extraction into an ethanol/diethyl ether/ammonia solvent system, the organic layer is evaporated to near dryness under nitrogen in a water bath. The resulting residue is dissolved in an acetonitrile/hexane/saturated sodium chloride solvent system. The acetonitrile layer is removed and dried under nitrogen. Following dissolution in an acetonitrile/triethylamine solvent system, the residue is derivatised with trifluroacetic anhydride. The derivatised sample is mixed with acetonitrile, centrifuged, and the supernatant is transferred for analysis. Samples are analysed by isocratic HPLC with fluorescence detection (360 nm excitation/470 nm emission).

Linearity was demonstrated over a range from 0.78 ng/mL to 25 ng/mL. The Limit of Detection (LOD) was calculated statistically to be 0.02 ng/mL and the Limit of Quantification (LOQ) was identified as being 0.78 ng/mL, reflecting the ability to validate at this concentration but not at the lower 0.39 ng/mL concentration. The accuracy and precision were 99% and ≤13.5%, respectively. The percentage recovery was 88% at the LOQ, declining to 65% at the 25 ng/mL concentration. Specificity of the method in the presence of other veterinary drugs was not described and there are no data demonstrating the stability of the analyte with storage.

Another, very similar method was evaluated by the 54th meeting (JECFA, 2000). The report estimated the performance range to be 5-50 µg/kg. Although a description of the analytical method was not submitted for the current evaluation, data supporting the statistically calculated LOD of 0.1 ng/mL were provided. Because the LOQ was defined *a priori* to be 10 times LOD, the LOQ for this assay was established at 1 ng/mL. The validating laboratory obtained a recovery of 87% and precision of 3% at the LOQ.

APPRAISAL

The Committee reviewed information on the LOD and LOQ of two HPLC methods for the determination of ivermectin in milk.

The Committee concluded that the methods could be recommended for routine monitoring of milk samples for ivermectin. However, because both analytical methods use as internal standard, avermectin B_{1a}, that is a component of an approved veterinary drug, the Committee recognized the potential for contamination of the milk with avermectin B_{1a} prior to sampling.

The Committee recommended that the temporary MRL of 10 µg/kg for cattle milk, expressed as ivermectin B_{1a}, be made permanent.

REFERENCES

Chick, B. (2000). A field study to determine the profile of Ivermectin H_2B_{1a} in milk following the administration of a 5 mg/mL ivermectin pour-on formulation in lactating dairy cattle. Final Report. Study No. N1007. Norbrook Laboratories Limited, Northern Ireland.

JECFA (2000) Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition Paper, 41/13.