6. Ivermectin

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Addendum to the monographs prepared by the 36th, 40th, 54th and 58th Meetings of the Committee and published in *FAO Food and Nutrition Papers* 41/3, 41/5, 41/13 and 41/14.

Background

Ivermectin is widely used as a broad-spectrum antiparasitic drug against nematode and arthropod parasites in food-producing animals. In human medicine, it is used mainly for the treatment of onchocerciasis. Ivermectin was previously considered by the Committee at its 36th, 40th, 44th and 54th Meetings. At its 40th Meeting, the Committee established an ADI of 0–1 µg/kg bw and recommended MRLs of 100 µg/kg for bovine liver and 40µg/kg for bovine fat, determined as ivermectin B1a. The 21st Session of the CCRVDF requested that the 78th Meeting of the JECFA advise on whether it was possible to establish an MRL for bovine muscle (FAO/WHO, 2013).

Current evaluation

At the present meeting, the Committee reviewed residue depletion data contained in the monographs for ivermectin prepared by the 36th and 40th Meetings of the Committee, which contained the residue data used to recommend MRLs for bovine liver and fat by the 40th Meeting of the Committee. A depletion study using [^{14}C]ivermectin reviewed by the 36th Meeting of JECFA showed that total ivermectin residues in muscle tissues of cattle were 1 µg/kg at 28 days following administration of a dose of 0.3 mg/kg bw by subcutaneous administration (FAO, 1991). The monograph also reported a study using unlabelled ivermectin in which cattle (approx. 260 kg bw) were administered ivermectin at 0.3 mg/kg bw by subcutaneous (s.c.) injection. In this study, no residues of ivermectin were detected at 28 days following treatment. The monograph reported that analytical methodology used for determination of the marker residue, ivermectin B1a, was based on high performance liquid chromatography with fluorescence detection (HPLC/FL), with a linear range of 5-60 µg/kg.

Subsequently, it was found that ivermectin residues were more persistent in heavy cattle (bw 450 kg) and an additional residue depletion study was reviewed by the 40th Meeting of the Committee (FAO, 1993). In this study, using cattle weighing 297 to 401 kg at time of drug administration, each animal received an s.c. injection of 0.3 mg/kg bw. Tissue samples were analysed using an improved HPLC/FL method with a limit of detection (LOD) of 1 µg/kg. At 28 days following treatment, there were detectable residues in muscle tissue, estimated at 1 µg/kg (the LOD). Typically, the limit of quantitation (LOQ) is approximately 3 times higher than the LOD, that is, the residues detected in the study were detectable, but below the LOQ and therefore not truly quantifiable. Based on the data considered by the 36th and 40th Meetings of the Committee, MRLs were recommended by the 40th Meeting of the Committee for bovine liver and fat, the two tissues in which greatest residues were detected. No MRLs were recommended for bovine muscle and kidney, although there were depletion data for both tissues, and concentrations of ivermectin in kidney and muscle were included in the calculation of the TMDI. The current Committee therefore concluded that it might be possible for JECFA to recommend an MRL for bovine muscle based on the existing summarized residue depletion data contained in the monographs, using the LOQ of current analytical methodology as a basis for an MRL for bovine muscle.

The LOQ of HPLC/FL methods, such as the method used in the depletion study considered by the 40th meeting of the Committee, remains similar today. However, many residue control laboratories now use a multi-residue method for ivermectin and related compounds, based on high performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). An analytical method for monitoring residues of ivermectin B1a in bovine muscle based on this technology was received and reviewed by the Committee.

Methods of analysis

Preliminary considerations

Muscle is frequently selected for residue surveillance of food of animal origin because it is a primary commodity in trade (Cooper *et al.*, 2012). The majority of assays are based on the extraction of ivermectin using acetonitrile with clean-up on C8, C18 or HLB sorbents (Danaher *et al.*, 2006, 2012). The use of LC gradient is recommended in analytical methods to reduce matrix carry-over peaks, particularly for fatty tissue. Similarly, single-residue methods have been applied for measuring ivermectin in the edible tissues of treated sheep (Nunez *et al.*, 2007). Immunoaffinity chromatography (IAC)-based clean-up procedures have been developed for the isolation of ivermectin from bovine tissues prior to HPLC-fluorimetry (He *et al.*, 2005) and LC-MS/MS (Hou *et al.*, 2006). Samples were simply extracted with acetonitrile and purified by C8 SPE prior to LC-MS/MS analysis. Another method was reported where samples were extracted using an acetone aqueous ammonia mixture and partitioned into iso-octane (Inoue *et al.*, 2009). Extracts were concentrated, re-suspended in n-hexane and partitioned into acetonitrile prior to LC-MS/MS analysis. Ivermectin residues are highly stable under a range of conditions (Danaher *et al.*, 2012; Cooper *et al.*, 2011). However, residues in minced meat submitted to cooking and frying diminished 45 and 50%, respectively (Slanina *et al.*, 1989; Rose, Shearer and Farrington, 1996).

Method principle

The TEAGASC laboratory (Dublin, Republic of Ireland) provided a validated method based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for consideration by the Committee (Danaher, 2013). The method has been implemented in an ISO-17025 accredited laboratory. The sample is prepared for analysis using a modified QuEChERS method (*Quick Easy Cheap Effective Rugged Safe*). The sample is extracted by shaking in acetonitrile, MgSO4 and NaCl before being cleaned up by dispersive SPE, using C18 and MgSO4, concentrated, filtered and transferred to a HPLC vial. Ivermectin residues are determined by ultra-performance liquid chromatography on a reverse phase C18 column (1.8 micron particle size, flow rate of 0.6 ml/min, column temperature set at 60°C) and coupled via an electrospray interface operating in the positive mode to a triple quadrupole mass spectrometer (QqQ). The signal acquisition is programmed in the selected reaction monitoring mode (SRM). Two transitions are monitored for ivermectin B1a, i.e. 890.4>305.2 (cone voltage 15 V, collision energy 25 eV) and 890.4>567.0 (cone voltage 15 V, collision energy 13 eV). An internal standard (selamectine, structural analogue) is used.

Method performance

The method has been validated for selectivity/specificity, linearity of calibration curve, working range, detection limit, decision limit (CCα), detection capability (CCβ), recovery, within-laboratory repeatability and within-laboratory reproducibility. The calibration curve was derived by fortifying negative muscle samples across a 1 to 50 µg/kg concentration range for ivermectin B1a. Value for the goodness of fit (R2) was 0.987. The working ranges are derived from the range of the calibration curve, i.e. 1 to 50 µg/kg. For each assay, 18 blank tissue samples were fortified at three different levels: 2, 3 and 4 µg/kg (n = 6 replicates

each concentration). These concentrations represent 1, 1.5 and 2 times the second-lowest calibration level, i.e. 2 µg/kg, considered as the minimum concentration of analyte that the method can determine with acceptable accuracy and precision. To establish the selectivity/specificity of the method, twenty muscle samples (non-fortified) were analysed. No interfering peaks were observed at the retention time of ivermectin in any of these samples. The LOD (defined by the laboratory as the limit above which it can be concluded with an error of probability of 1% that a sample contains the analyte) was 0.8 µg/kg. The limit of decision (CCα) and the detection capability (CCβ) were calculated on the basis of the spectrometric signals observed at the second-lowest calibration level, i.e. 2 µg/kg. CCα and CCβ were 2.8 and 3.9 µg/kg, respectively. The recovery (expressed as a percentage) was calculated by analysing 18 blank muscle samples fortified at three different concentrations, 2, 3 and 4 µg/kg (n = 6, each concentration) operated on three different days in one experiment and by three different operators in a second experiment. At 2 µg/kg, the overall recoveries calculated for three days and three different operators were 99.2% and 105.2%, respectively. The overall recovery was judged to meet the requirements of the CAC/GL 71-2009 guideline (FAO/WHO, 2012). For estimation of the precision in term of within-laboratory variation (repeatability), the same samples were used and the variation in recovery was presented as a relative standard deviation (RSD) by dividing the standard deviation by the mean concentration. At 2 µg/kg, 3 µg/kg and 4 µg/kg, the precision was 6.5%, 12.0% and 6.4%, respectively. The precision in terms of within-laboratory reproducibility was calculated at three fortification levels from the results of three different analysts. The results obtained were presented as RSD (%). At 2 µg/kg, 3 µg/kg and 4 µg/kg, the precision was 25.3%, 22.5% and 18.3%, respectively. Precision expressed in terms of within-laboratory repeatability and intermediary reproducibility was judged adequate to meet the requirements of guideline CAC/GL 71-2009.

The Committee assessed the validation data against the requirements as published in the Codex guidelines for analytical methods for residue control (CAC/GL71-2009). In particular, the Committee reviewed information on the LOD (0.8 µg/kg) and LOQ (2 µg/kg) of the submitted LC-MS/MS method for the determination of ivermectin B1a in muscle. The Committee concluded that the analytical method can be recommended for regulatory monitoring of ivermectin B1a residues in muscle samples.

Maximum Residue Limits

In recommending MRLs for ivermectin in cattle muscle, the Committee considered the following factors:

- A new compliant fully validated LC-MS/MS method complete with adequate performance factors and method validation was provided that was considered suitable for routine monitoring of ivermectin B1a as marker residue.
- The analytical method has been validated for use in bovine muscle, with an LOQ of 2 µg/kg.
- The radiolabel study considered by the 36th Meeting of the Committee demonstrated that the total residue of ivermectin in muscle at 28 days was 1 µg/kg.
- The depletion study considered by the 40th Meeting of the Committee based on which MRLs were recommended for bovine fat and liver demonstrated that residues of the marker residue in bovine muscle at 28 days, the time-point at which MRLs were recommended for bovine fat and liver, were approximately 1 µg/kg, using an analytical method with an LOD of 1 µg/kg.

The Committee recommended an MRL of 4 µg/kg for cattle muscle determined as ivermectin B1a, based on 2 × LOQ of the analytical method. The dietary intake calculation prepared by the 40th Meeting of the Committee included an estimate of the potential intake

from muscle, based on the concentrations of total residue reported from the radiolabel study.

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