RESIDUE DEPLETION OF IVERMECTIN, MOXIDECTIN AND DORAMECTIN IN CATTLE TISSUES

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Background

The use of antiparasitic drugs as chemotherapeutic agents in animal production has increased in the last decade. Antiparasitics, su as endectocides, have became an integral part of the livestock industry, acting to prevent parasitic diseases (Campbell & Benz,198 However, because of the nature of these compounds (Fisher,M. & Helmut, M. ,1992), residues of these drugs in food derived from treat animals could pose a health treat to consumers. Regulatory agencies have established withdrawal periods for treated animals prior slaughter and also maximun residue levels allowable in tissues (FAO /WHO, 1993; FAO /WHO, 1996).The principal members of th family are ivermectin (IVM), moxidectin (MOX) and doramectin (DOR). The structures of the three molecules are closely related. For t three molecules, the unaltered drug was the major residue in the liver, fat, muscle and kidney tissue of all species studied at all points Tissue residue depletion studies have been reported by the sponsors for the three compounds in separated trials (FAO /WHO 1993; FA /WHO 1996) with large differences in animals characteristics, production systems, doses employed, analytical methodology, etc. Being th information obtained in non standarized experimental conditions, we can't compare the residue performance of the three molecules equal basis.

Objective

The present study was undertaken in order to determine the distribution and depletion of residues of ivermectin, moxidectin and doramectin in steer food tissues in the same trial under standarized conditions and using an unique MSPD analytical procedure with very low detection levels.

Methods

Thirty male Hereford calves (130-140 kg) were used. The animals were randomly allocated in three groups (n=10), two non-treat calves used as controls. Each group was treated with the commercially available formulations of ivermectin, moxidectin and doramectin b s.c. injections at the same dose rate (200 ug/kg) and tissue samples were taken at slaugter (8, 18, 28, 38 and 48 days post-treatment) fro fat, muscle, liver and kidney.

Analytical procedure - The extraction and cleaned-up procedure is based on the matrix solid phase dispersion technique (MSPD and was achieved by using a previously described process (Alvinerie et al., 1996). The molecules are derivatized as previously describe (Alvinerie et al., 1996) to give intensely fluorent compounds that were quantified by HPLC. The HPLC system consisted of a HP 10 delivery pump, a model 1046A fluorescence detector connected to a HP1050 HPLC Chemstation (Hewlett-Packard, Buenos Aire Argentine). The separation was carried out on a HP Spherisorb ODS 2 column (5µ, 250 x 4.6 mm i.d.) maintained a 30°C. The mobil phase of methanol:water 95:5 was pumped at a flow rate of 1.8 ml/min. Under these conditions, the typical retention times for ivermecti moxidectin and doramectin were 3.4, 3.9 y 5.0 min. respectively. The detector was fixed at an excitation wavelength of 383 nm and a emission wavelength of 440 nm (gain=15).

Quality parameters of methodology - Calibration graphs for the three molecules in the range 1-100 ng were prepared using drug-free calv tissues. The fortified tissue samples were taken through the procedure and assayed by HPLC. Calibration graphs were constructed using th peak area as a function of analyte concentration and least-squares regression analysis was used to determine slope. The correlatio coefficient generally exceeded 0.990. The limits of quantification (LOQ) were 5.0, 5.0 and 6.0 ppb for the ivermectin, moxidectin an doramectin respectively, being the limits of detection (LOD) 1.5, 1.0, and 2.0 ppb for the same molecules. The extraction recoveries wer tested using samples spiked at 20 ppb and were in excess of 70 %. The inter-assay precision of the method, expressed as the RSD, wa below 8 %. Results are presented in Table 1.

Results an discussions

Residue depletion for the main molecules is presented in figures 1, 2 and 3 .These values are the average levels found for tw animals at each point, each tissue assayed in triplicate. Figures 4 and 5 shows the comparative depletion for the three molecules in liver an fat. Taking into account these results, we can make the following considerations:

- The residues show the same distribution pattern for the three molecules. They are highest in fat and liver and lowest in muscle and kidne (Figures 1,2,3) at all times post-treatment, even if the kinetics are different. - Fat residues are clearly highest for the moxidectin, middle fo the doramectin and lowest for the ivermectin, indicating a higher accumulation of moxidectin as unchanged drug in fat.(Figure 4). Th accumulation of the endectocides in fat as unchanged drugs can explained the sustained action of these molecules. - Comparing live residues to fat residues, fat residues are higher than liver ones at all times post-treatment for the moxidectin. For the ivermectin, live residues are higher than fat residues until 28 days post-treatment and for doramectin, only to 8 days post-treatment. This shows a differen kinetic for these molecules in the different tissues.

- Comparing the depletion of liver residue (Figure 5) , we marked: a) the ivermectin shows the higher residues at 8 days withdrawal dropping very quickly to reach levels lower than the other molecules with time b) doramectin shows a similar profile but a more attenuate slope, with residue values lower than ivermectin at 8 days post-treatment and higher than ivermectin at longer times c) moxidectin show the minor values at all times.

The differences we have found for the three molecules in tissue distribution and elimination kinetic, probably reflect their differences i pharmacokinetics and biotransformation, (Alvinerie & Galtier, 1997; Wicks et al., 1993; Chiu et al, 1988; Zulalian et al.,1994).Being th moxidectin the more lipophilic one and the more biotransformated, the doramectin presents an intermediate behavior compared to th others endectocides.

The comparison between the residue levels measured in this trial and the maximum limits of residues (LMR) established for these molecules in edible tissues shows that residue levels are below the LMR at 18 days in liver and at 28 days for the other tissues for ivermectin, at 28 days and 8 days respectively for moxidectin and at 18 days in liver and kidney and 28 days for fat and muscle for doramectin.

In spite of differences between the endectocides, residue levels make meat available to be consumed at 28 days post-treatment.

Conclutions

On the basis of these results obtained in a same trial under standarized experimental conditions, we can conclude:

- The results obtained concerning the distribution and residue elimination kinetic of each endectocide are in good agreement with previously reported in individual experiments. In addition to the metabolism that can explain partially the differences between molecules, these results can provide information for better understanding the action of these endectocides, particularly its long permanence in the body that it is attributed to its long persistence in fat tissue.
- The 35 days withdrawal time generally accorded to the treated animals is time enough to guarantee an intake without risk of meat of endectocides treated animals because residue levels are under the Codex Committee recommended MRLs for the three compounds.

LITERATURE CITED

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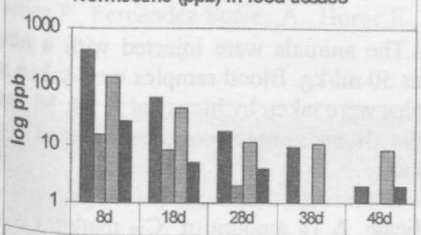
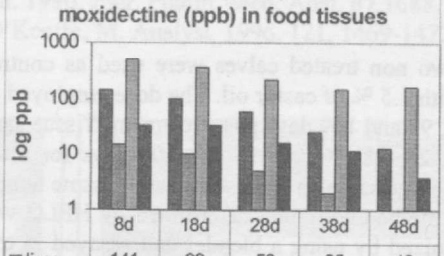
Figure 1. Residue depletion of ivermectine (ppb) in food tissues



	8d	18d	28d	38d	48d
liver	434	68	17	9	2
muscle	15	8	2	0	0
fat	148	44	11	10,5	8
kidney	27	5	4	0	2

days p.t.

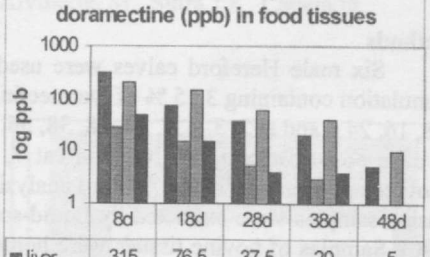
Figure 2. Residue depletion of moxidectine (ppb) in food tissues



	8d	18d	28d	38d	48d
liver	141	99	58	25	13
muscle	15	10	5	2	0
fat	487	357	219	140	161
kidney	32	32	16	12	4

days p.t.

Figure 3. Residue depletion of doramectine (ppb) in food tissues



	8d	18d	28d	38d	48d
liver	315	76,5	37,5	20	5
muscle	28	15	5	3	0
fat	210	150	59	40	10
kidney	50	15,5	4	4	0

days p.t.

Table 1. Recovery (%) of IVM, MOX and DOR from fortified tissue samples (n=3, 20ppb)

Tissue	IVM	MOX	DOR
Liver	93.3 (6.2)	96.3 (7.0)	95.0 (5.0)
Muscle	85.5 (8.0)	90.0 (6.5)	88.3 (4.7)
Fat	70.0 (8.0)	75.0 (7.0)	70.4 (8.2)
Kidney	80.0 (5.0)	82.3 (4.8)	80.3 (6.0)

RSD values are in parentheses

Figure 4 - Residue depletion of Ivermectin, Doramectin and Moxidectin (ppb) in liver

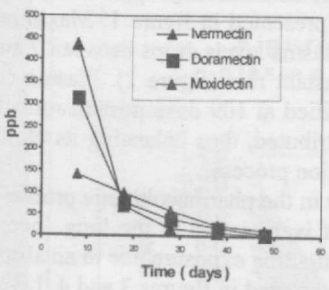


Figure 5 - Residue depletion of Ivermectin, Doramectin and Moxidectin (ppb) in fat