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Rapid screening method for ivermectin residue detection in cattle muscle and liver by liquid chromatography with UV detection

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Abstract

A rapid screening method for the determination of residue of ivermectin in edible bovine tissue and liver is described The analyte is extracted from the tissue with a methanol–water mixture The clean-up procedure is based on solid phase extraction on a Sep-Pak C_{18} cartridge Analysis is performed by isocratic elution with methanol–acetonitrile–water (9 9 2, v/v/v) on a Novapak C_{18} 5-μm column and UV–diode array spectrophotometric detection at 254 nm The average recovery from spiked bovine muscle was 78 6% (S D = 15 0%) and from spiked liver sample 78 5% (S D = 10 9%) in the concentration range 14–275 $\mu g\ kg^{-1}$ The method has a detection limit of 5–10 $\mu g\ kg^{-1}$

Keywords Liquid chromatography, Bovine tissue, Cattle, Ivermectin, Liver tissue, Muscle tissue

Ivermectin is a very potent broad spectrum antiparasitic drug It is a mixture of two homologous macrocyclic lactone disaccharides, containing not less than 80%, 22,23-dihydroavermectin B_{1a} (H_2B_{1a}) and not more than 20% 22,23-dihydroavermectin B_{1b} (H_2B_{1b}) (Fig 1) The drug is effective in very low dosage against nematodes and arthropod parasites in cattle [1] and has been widely used in the treatment of endo- and ectoparasites in sheep, horses and cattle Since 1991 Spain includes the analytical control of ivermectin residues in the National Residue Program Therefore, a rapid screening method is needed for the analysis of meat tissues and liver

Tolerance levels (25–100 $\mu g\ kg^{-1}$) are known in the U S A Residue Program, where the detection levels of the analytical method have to be able to detect 15–20 $\mu g\ kg^{-1}$ of ivermectin in liver depending on the species Recently the E C published their residues levels and put the tolerance levels for ivermectin at 15 $\mu g\ kg^{-1}$ in meat and 20 $\mu g\ kg^{-1}$ in liver [2]

Liver is the target tissue for residue control as was shown by Prabhu et al [1] who studied the ivermectin distribution in fat, liver, muscle and kidney after oral and in-feed administration of ivermectin in swine Although the metabolism of ivermectin is different in swine from that in cattle, the unaltered parent drug was shown to be the major residue component in both animals [3]

Several analytical procedures have been used to measure ivermectin in animal tissues The first reported methods for determining avermectins in animal plasma [4,5] were based on UV detection, the first after Florisil clean-up of the plasma, and the second method using a tedious liquid–liquid-

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extraction clean-up followed by normal phase liquid chromatography (LC) A very rapid reversed-phase method for determining concentrations in serum, also with UV detection uses two cartridges (C_{18} and silica) for clean-up of the sample which makes the procedure more rapid, but also more expensive [6] Methods for the determination of ivermectin in tissues have been reported using LC with fluorescence detection after clean-up procedures based on two cartridges [7] or several liquid–liquid solvent partition steps [1] followed by derivatization of ivermectin to a fluorescent product and separation and quantitation by LC with fluorescence detection

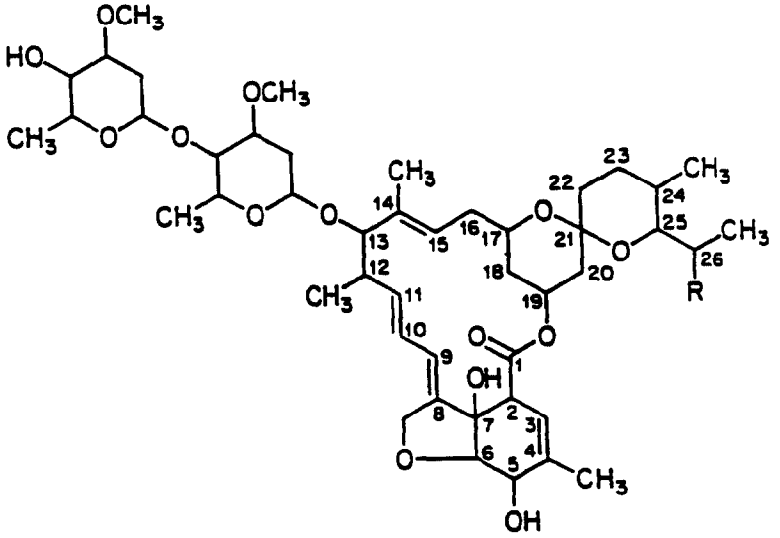
The method reported here is based on extraction of ivermectin with acetonitrile and clean-up of the extract with a C_{18} cartridge The residue, evaporated till dryness, is injected in an LC system with detection of absorbance at 254 nm In the described method, ivermectin will be used as the drug name However, dihydroavermectin H_2B_{1a} has been used in residue studies because it is the major compound and metabolized at a lower rate than H_2B_{1b}

EXPERIMENTAL

Apparatus and reagents

Ivermectin in glycerol formal with 1 38% H_2B_{1a} and 0 21% H_2B_{1b} was obtained from Merck, Sharp and Dohme (Rahway, NJ) Acetonitrile (Panreac) and methanol (Romil, Loughborough) was HPLC grade Sep-Pak C_{18} cartridges (Millipore-Waters, Milford, MA) were used Water was Milli-Q (Millipore-Waters) deionized Ultraturrax (Janke-Henkel, Heidelberg) was used for homogenization

The LC system (Millipore-Waters) was composed of a Model 510 dual liquid chromatographic pump, a universal injector (model U6K), a Model 441 UV detector of fixed wavelength (254 nm) (Waters) with a Data Module integrator (Waters) For confirmation purposes, the photodiode array spectrophotometric detector with the corresponding Model 990 printer–plotter (Millipore-Waters) was used The chromatographic column used throughout this work, at room temperature, was packed with Novapak 5-μm C_{18} (150 × 3 9 mm i d) (Millipore-Waters) and acetoni-



I: R = CH_3CH_2
II: R = CH_3

Fig 1 Structure of (I) 22,23-dihydroavermectin B_{1a} and (II) 22,23-dihydroavermectin B_{1b}

trile–methanol–water (9 9 2, v/v/v) was the mobile phase The flow-rate was 1 ml min^{-1}

Extraction and clean-up of samples

Samples of bovine muscle and liver tissue were minced and mixed in a Braun homogenizer A 5-g mass of the homogenate was placed into a screw-topped glass centrifuge bottle (250 ml), 3 5 ml of water and 45 ml of acetonitrile were added and the content was thoroughly mixed using an ultra-turrax The bottles were centrifuged at 2800 *g* for 10 min and supernatants were transferred to round-bottomed amber glass flasks and extractions were repeated with a fresh water–acetonitrile mixture The combined supernatants were evaporated to ca 6 ml in a rotary evaporator with vacuum and a waterbath temperature of 60°C The aqueous suspensions were applied to Sep-Pak C_{18} cartridges (Millipore-Waters), which were pretreated with 4 ml of acetonitrile and 4 ml of acetonitrile–water (1 1) using plastic disposable syringes The flow-rate of solvents through Sep-Pak should be about 10 ml min^{-1} The cartridges were eluted with 3 + 2 ml of acetonitrile and the eluate, combined in screw-topped amber glass tubes, was evaporated to dryness at 50°C

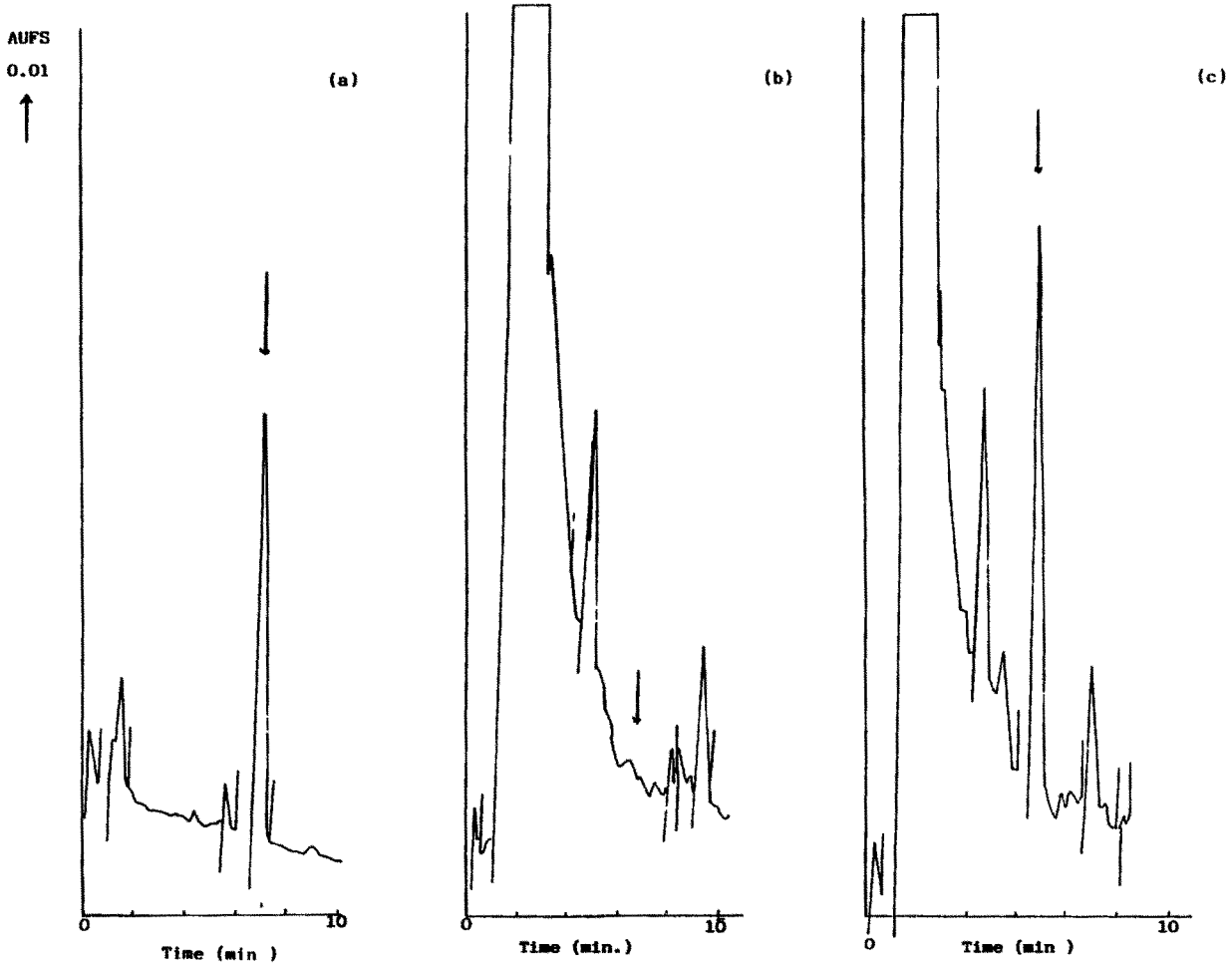


Fig 2 Typical chromatograms of (a) standard ivermectin (138 ng), (b) blank and (c) spiked (207 $\mu g\ kg^{-1}$) muscle sample LC conditions as described in Experimental

under a gentle stream of nitrogen The dry residue was dissolved in 200 μl of methanol

Detection and identification by LC

A standard stock solution of 13 8 ng ml^{-1} dihydroavermectin B_{1a} was prepared in methanol (1 1000 dilution of the commercial standard) and stored in a freezer (−20°C) A working standard solution was prepared diluting the standard solution ten-fold (1 38 ng μl^{-1}) in mobile phase With both solutions, a standard curve was made in the concentration range of 6–250 ng per injection by plotting peak height at 0 01 absorbance full scale versus injected amount in the chromatographic system described

The extraction method was validated with control and fortified samples of bovine meat and liver in the 10–200 $\mu g\ kg^{-1}$ range

The identity of peaks at the ivermectin retention time may be confirmed with a diode array spectrophotometric detector, using a wavelength range of 220–300 nm

RESULTS AND DISCUSSION

Previously reported methods for the determination of ivermectin in tissue are sensitive and specifically based on LC separation of fluorescent ivermectin derivatives and fluorescence detection, or unspecific, based on LC with UV detection, generally with complicated extraction and time consuming clean-up procedures

As ivermectin is quantitatively extractable with organic solvents from liver and muscle tissue, the proposed method here uses extraction of the sample with water–acetonitrile, which does precipitate the proteins

First of all recovery of ivermectin in the solid phase clean-up step was tested As was described in Ref 7, the residual silanol groups in reversed-phase material could cause irreversible absorption of ivermectin on the cartridges, however, we obtained recoveries of ivermectin H_2B_{1a} of 95–100% after its purification on Sep-Pak C_{18} in the described conditions The linearity of the detector response at 254 nm was controlled, injecting various amounts of ivermectin, in the range of 6–120 ng

The regression line passed close to the origen, with a slight negative intercept $y = 0\,96x - 0\,46$, where x = ng of injected ivermectin, and y = peak height in mm at 0 01 absorbance full scale (= 235 mm) The correlation coefficient was 0 9955 (n = 16) Peak-height measurement was preferred rather than peak-area because not always a good peak integration was obtained, as in this screening method various other peaks may interfere in a

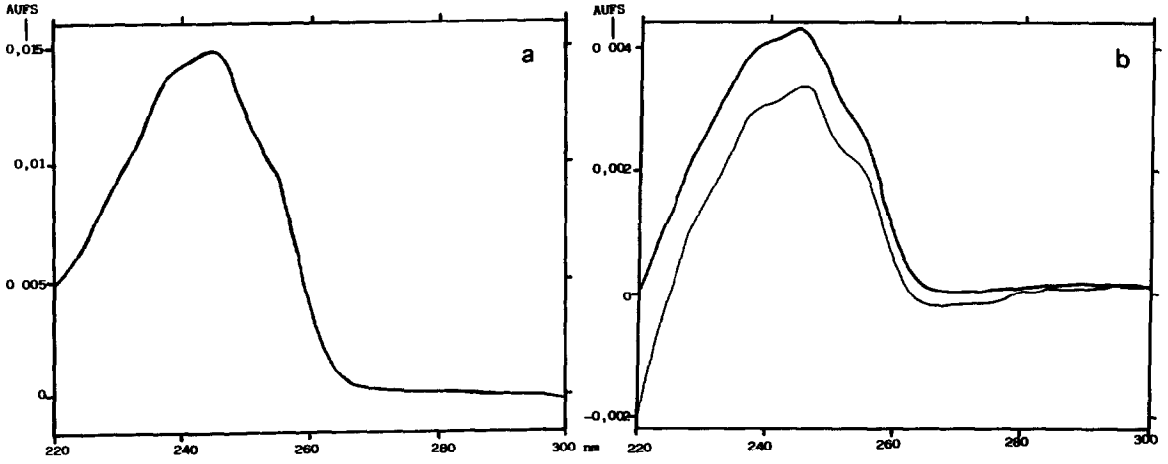


Fig 3 Spectra of (a) standard ivermectin (138 ng) and (b) of the ivermectin peak of muscle samples spiked with 69 and 138 $\mu g\ kg^{-1}$

good integration In Fig 2a, ivermectin H_2B_{1b} can also be indicated in the chromatogram of the standard solution

Recovery assays in bovine muscle samples were carried out with spiked samples at the 14–275 μg kg^{-1} level Results are presented in Table 1, and as can be seen the mean recovery value ($\pm$S D) of 26 determinations is 78 6 $\pm$ 15 0% A typical chromatogram of standard ivermectin (138 ng), blank muscle sample and spiked (207 μg kg^{-1}) muscle sample is shown in Fig 2, and as may be observed, the detection limit of the method for ivermectin in meat samples is about 5 μg kg^{-1} However, confirmation of the presence of ivermectin using diode array spectrophotometric detection is not possible at this level A satisfactory spectrum can only be obtained at levels higher than 20–30 μg kg^{-1} An example is shown in Fig 3

TABLE 1

Recovery of ivermectin added to muscle tissue at the 276 μg kg^{-1} level [a]

Tissue	(μg Kg^{-1}) Spiked	n	Recovery (%)	S D (%)
Blank muscle tissue	–	5	–	–
Muscle tissue	14	4	65	12
	28	4	74	10
	69	5	79	11
	138	5	85	15
	276	8	89	12
Average			78 6	15 0

[a] Limit of detection = 5 μg kg^{-1}

Recovery assays were also carried out with

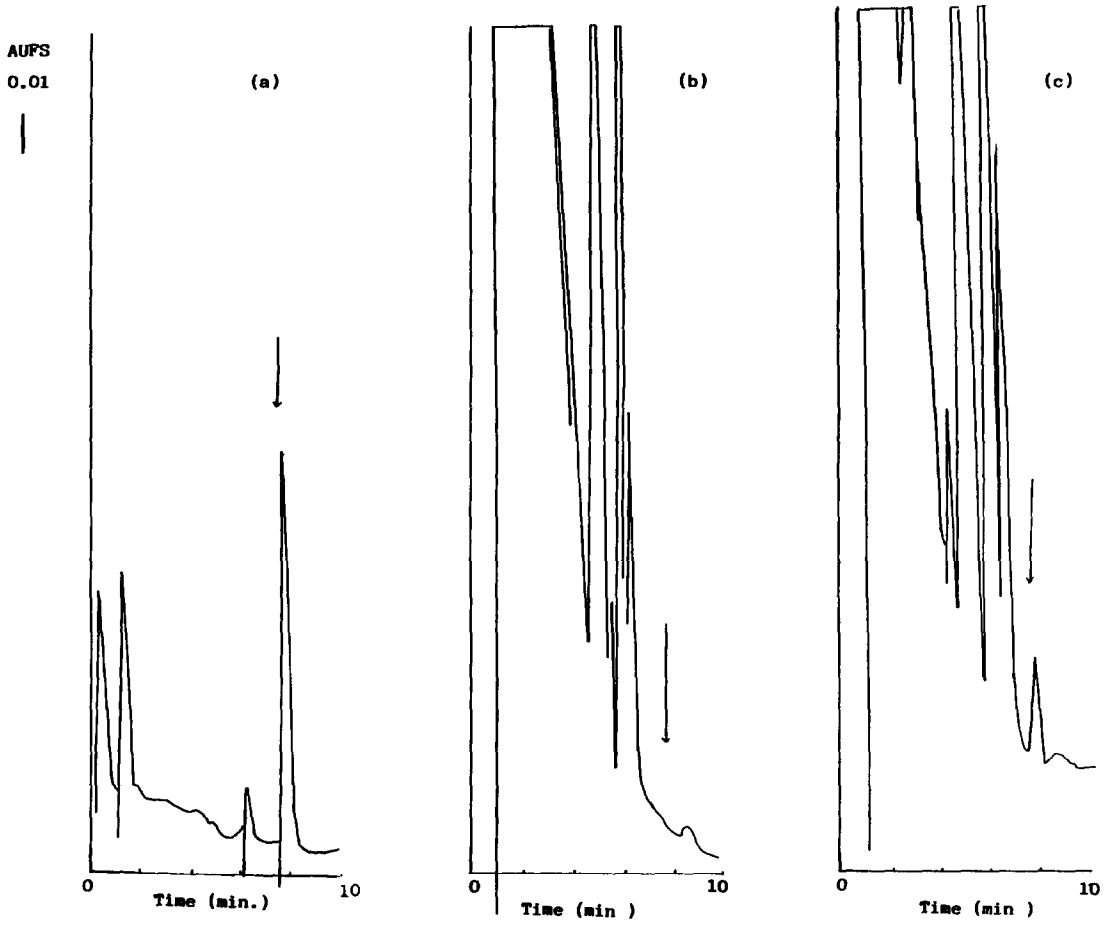


Fig 4 Typical chromatograms of (a) standard ivermectin (69 ng), (b) blank liver sample and (c) spiked (41 μg kg^{-1}) liver sample

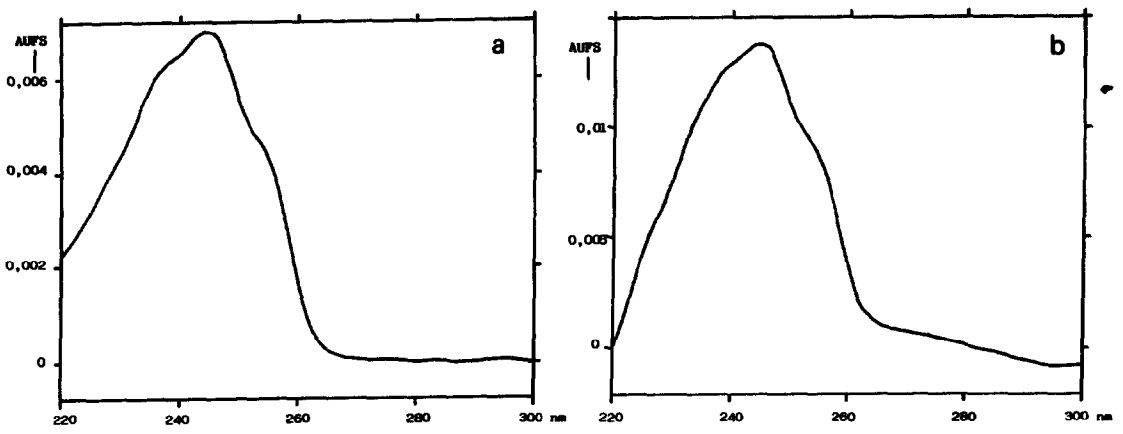


Fig 5 Spectra of (a) standard ivermectin (69 ng) and (b) liver sample spiked with 276 μg kg^{-1} ivermectin

liver sample, the target tissue of ivermectin residue control Table 2 shows the obtained values of 25 determinations yielding a mean recovery of 70 5 ± 10 9%, at the range of 25–275 μg kg^{-1} A typical chromatogram of standard ivermectin (69 ng), blank and spiked (41 μg kg^{-1}) liver sample is shown in Fig 4, from which may be concluded that the detection limit of this method for liver samples is 5–10 μg kg^{-1}. Confirmation by diode array spectrophotometry using the same LC system may be achieved at levels higher than 50 μg kg^{-1}

An example of peak confirmation is shown in Fig 5, where a spectrum of directly injected ivermectin (69 ng) is compaired with the ivermectin peak spectrum of a spiked liver sample (276 μg kg^{-1})

TABLE 2

Recovery of ivermectin added to liver tissue at the 28–276 μg kg^{-1} level [a]

Tissue	Spiked (μg kg^{-1})	n	Recovery (%)	S D (%)
Blank liver	–	5	–	–
Liver tissue	28	4	80	11
	69	10	72	6
	138	6	80	12
	276	5	89	5
Average			78 5	10 9

[a] Limit of detection = 5–10 μg kg^{-1}

The analytical method was successfully applied to samples provided by the Inspection Service for the National Residue Program Till now, no positive samples were found The proposed method may be used as screening method for ivermectin residue detection in meat and liver samples at the 5–10 μg kg^{-1} level and at levels higher than 20–50 μg kg^{-1} (meat and liver) peaks may be confirmed by diode array spectrophotometric detection, compairing the standard spectrum with those of the suspicious peaks

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