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DETERMINATION OF BENZIMIDAZOLE ANTHELMINTICS IN MEAT SAMPLES

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SUMMARY

A procedure for the detection of eight benzimidazole anthelmintics in meat samples using high-performance liquid chromatography is described. The limits of detection are in the range 20–50 μg/kg with a recovery of 66–87%. Chromatography is performed on an octadecylsilane column using mobile phases of acetonitrile–water with an ion-pair reagent, with UV detection. For verification of positive results, the drug substances are derivatized to methyl or pentafluorobenzyl derivatives suitable for detection by gas chromatography–mass spectrometry in the electron-impact or positive or negative chemical-ionization mode.

INTRODUCTION

Benzimidazoles are of importance as anthelmintics. As some members of this class of drugs have shown embryotoxic properties with some animal species, the analysis of residues was of major interest. Also, for the establishment and control of tolerance values a method for monitoring residues of benzimidazoles in meat was necessary. There have been many reports of the detection of particular benzimidazoles in plasma, serum, body fluids[1–4] and tissues[5–9] using high-performance liquid chromatography (HPLC) with UV or fluorescence detection. At present no method is available for the determination and subsequent verification of all benzimidazoles registered in Switzerland (see Fig. 1) in a single procedure. Methods for the detection of benzimidazoles and related compounds by gas chromatography (GC) and verification of the results by GC–mass spectrometry (GC–MS) have been described for thiabendazole (**7**) as the pentafluorobenzoyl derivative[10], the tetramethylsilyl derivative, which decomposes to the isocyanate[11], the methyl derivative[12] or the pentafluorobenzyl derivative[13]. Many methods have been developed for the determination of the carbamate pesticides[13–15], which are related to the "carbamate-type" benzimidazoles (see Fig. 1).

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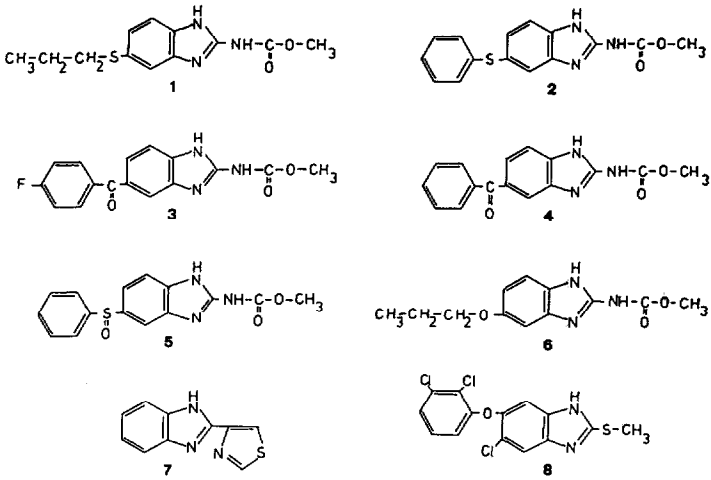


No.	DCI name	Chemical name
1	Albendazole	Methyl 5-propylthio-2-benzimidazolecarbamate
2	Fenbendazole	Methyl 5-phenylthio-2-benzimidazolecarbamate
3	Flubendazole	Methyl 5-(4-fluorobenzoyl)-2-benzimidazolecarbamate
4	Mebendazole	Methyl 5-benzoyl-2-benzimidazolecarbamate
5	Oxfendazole	Methyl 5-phenylsulfinyl-2-benzimidazolecarbamate
6	Oxibendazole	Methyl 5-propoxy-2-benzimidazolecarbamate
7	Thiabendazole[a]	2-(4-Thiazolyl)benzimidazole
8	Triclabendazole	5-Chloro-6-(2,3-dichlorophenoxy)-2-methylthiobenzimidazole

Fig. 1. Benzimidazoles investigated. DCI stands for denominatio communis internationalis, *i.e.* international non-proprietary name. [a] No longer registered in Switzerland.

In this paper, an HPLC method is described, which was tested with different tissue and blood samples, including samples from pigs treated with fenbendazole (**2**).

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Hewlett-Packard (Palo Alto, CA, U.S.A.) 1081B isocratic solvent-delivery system, a Hewlett-Packard 79841/2 injector and autosampler, an Uvikon 722LC variable-wavelength spectrophotometric detector (Kontron, Zurich, Switzerland) and a Hewlett-Packard Model 3390A reporting integrator. The column was a Hewlett-Packard RP-18 (5 μm) type (200 × 4.6 mm I.D.), connected to a Kontron RP-18 Presat precolumn (40 × 4.6 mm I.D.) of particle size 25–40 μm.

The GC system was a Carlo Erba (Milan, Italy) HRGC 5160 gas chromatograph with a split–splitless injector and an NPD 40 nitrogen–phosphorus detector or a Perkin-Elmer (Norwalk, CT, U.S.A.) Sigma-2000 gas chromatograph with a split–splitless injector, a nitrogen–phosphorus detector and a Perkin-Elmer LCI100 computing integrator. GC was performed using OV-1-CB fused-silica capillary

columns (10 m × 0.25 mm I.D.; film thickness 0.25 μm; chemically bonded) (Macherey, Nagel & Co., Düren, F.R.G.) and helium as the carrier gas (flow-rate *ca.* 1 ml/min) with temperature programming (60°C for 0.5 min, increased from 60 to 150°C at 30°C/min and from 150 to 300°C at 6°C/min). The injector temperature was 270°C and the detector was set at 350°C. The injection mode was splitless with an injection volume of 1 μl.

The GC–MS system consisted of a Finnigan-MAT (San Jose, CA, U.S.A.) 5100 quadrupole GC–MS system, equipped with an interchangeable electron-impact–chemical-ionization (EI–CI) ion source and an INCOS data system with the PPINICI option. GC was performed on a DB-1 fused-silica column (30 m × 0.25 mm I.D.; film thickness 0.25 μm; chemically bonded) (J&W Scientific, Folsom, CA, U.S.A.) with helium as the carrier gas (flow-rate *ca.* 1 ml/min). The injector temperature and temperature program were as described above, the interface oven temperature was set at 300°C and the injection volume was 1 μl. The operating conditions for the mass spectrometer were as follows: direct interface, ionization voltage 70 eV, source temperature 250°C, mass range 50–750 amu, scan rate 480 amu/s, source pressure reading in positive or negative CI mode 1.6 mbar (reactant gas methane) and manifold pressure 10^{-7} bar.

Chemicals

The chemicals used were iodomethane (Fluka, Buchs, Switzerland), methane of 99.995% (v/v) purity (Carbagas, Berne, Switzerland), pentafluorobenzyl bromide (α-bromo-2,3,4,5,6-pentafluorotoluene) (Fluka), pentanesulphonate solution (PIC reagent B5) (Waters Assoc., Milford, MA, U.S.A.), phosphorus pentaoxide (Siccapent) (Merck, Darmstadt, F.R.G.), Sep-Pak C_{18} cartridges (Waters Assoc.), Sep-Pak Florisil cartridges (Waters Assoc.) and triethylamine (Fluka). Solvents such as acetone, acetonitrile, chloroform, dichloromethane, ethyl acetate, hexane, methanol and tetrahydrofuran were of Merck LiChrosolv or analytical-reagent grade. The reference substances albendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, thiabendazole and triclabendazole were kindly provided by the manufacturers.

Detection by HPLC

Operating conditions. Under normal conditions, mobile phase 1 (see Table I) was used; in order to detect or determine oxfendazole (**5**) in certain extracts mobile phase

TABLE I

MOBILE PHASES USED IN HPLC

Mobile phase No.	*Components*[a]	*Proportions (v/v)*
1	Acetonitrile–methanol–ion pair mixture	35:35:30
2	Acetonitrile–ion pair mixture	50:50
3	Acetonitrile–ion pair mixture–water	43:45:12

[a] Ion pair mixture: aqueous solution of 0.01 *M* pentasulphonate (PIC-B5) and 0.5% (m/v) triethylamine, adjusted to pH 3.5 with acetic acid (100%).

2 or 3 was used. When special matrix-dependent problems arose, these mobile phases were also used.

The mobile phase was filtered through a 0.45-μm filter (HVLP; Millipore, Bedford, MA, U.S.A.), degassed and pumped at a rate of 1.0 ml/min. The detector wavelength was set at 298 nm, the column temperature was ambient and the injection volume was 10 μl.

Standard solutions. Stock standard solutions of 10 mg of each of the benzimidazoles in 20 ml of methanol or tetrahydrofuran were prepared by dissolution with heating or in an ultrasonic bath. Working standard solutions were prepared by suitable dilution of the stock solutions with acetonitrile.

Extraction procedure. A 10-g amount of ground tissue was homogenized with 20 ml of acetonitrile in a Polytron mixer (Kinematica, Littau, Switzerland). The suspension was centrifuged at 4000 rpm (2.2 *g*) for 5 min and the supernatant was removed and saved (extract 1). The residue was treated again in the same manner with 12 ml of acetonitrile and 3 ml of water (extract 2). Extract 1 was defatted with 50 ml of hexane in a separating funnel, then extract 2 was treated in the same manner with the same hexane. The acetonitrile phases were pooled, and subsequently 20 ml of hexane and 3 g of sodium chloride were added and the mixture was vigorously stirred and centrifuged at 4000 rpm (2.2 *g*) for 10 min. Three phases of saturated sodium chloride solution, acetonitrile and hexane were formed, of which the hexane phase was discarded. Then 10 ml of dichloromethane were added and the mixture was stirred and centrifuged in the same way as above. The acetonitrile phase (the upper layer) was removed, dried over sodium sulphate and evaporated with a vacuum evaporator (Rotavapor; Büchi, Flawil, Switzerland) at 40°C to a volume of 0.5–0.2 ml, the sodium sulphate being rinsed with 3 ml of acetonitrile. The extract was pipetted onto a Sep-Pak C_{18} cartridge that had previously been rinsed with 10 ml of methanol and 10 ml of acetonitrile. The benzimidazoles were eluted with 3 ml of acetonitrile and the solution was collected in a conical vial and evaporated with a stream of nitrogen at 40°C to a volume of 0.2 ml. The extract was then pipetted onto a Sep-Pak Florisil cartridge that had previously been conditioned with chloroform–methanol–triethylamine (90:10:1, v/v/v). The benzimidazoles were eluted with 10 ml of the same mixture, collected in a conical centrifuge tube and evaporated to dryness with a stream of nitrogen at 40°C. The residue was dissolved in 0.5 ml of the appropriate mobile phase for HPLC (normally phase 1), treated in an ultrasonic bath for 5 min and centrifuged at 4000 rpm (2.2 *g*) for 10 min. The clear solution was transferred into a 2-ml HPLC autosampler vial and submitted to HPLC analysis.

The extraction procedure is summarized schematically in Fig. 2.

Chromatography. Aliquots (10 μl) of sample and standard solutions were injected by means of the autosampler. The amount of the benzimidazoles was determined by means of external standards.

Detection and confirmation by GC and GC–MS

Operating conditions. These are described under *Instrumentation.*

Standard solutions. The standard solutions were prepared by dilution of the appropriate stock solutions of the benzimidazoles with acetone. Aliquots were submitted directly to the derivatization procedure as described below.

Extraction procedure. The extraction procedure is described under *Detection by*

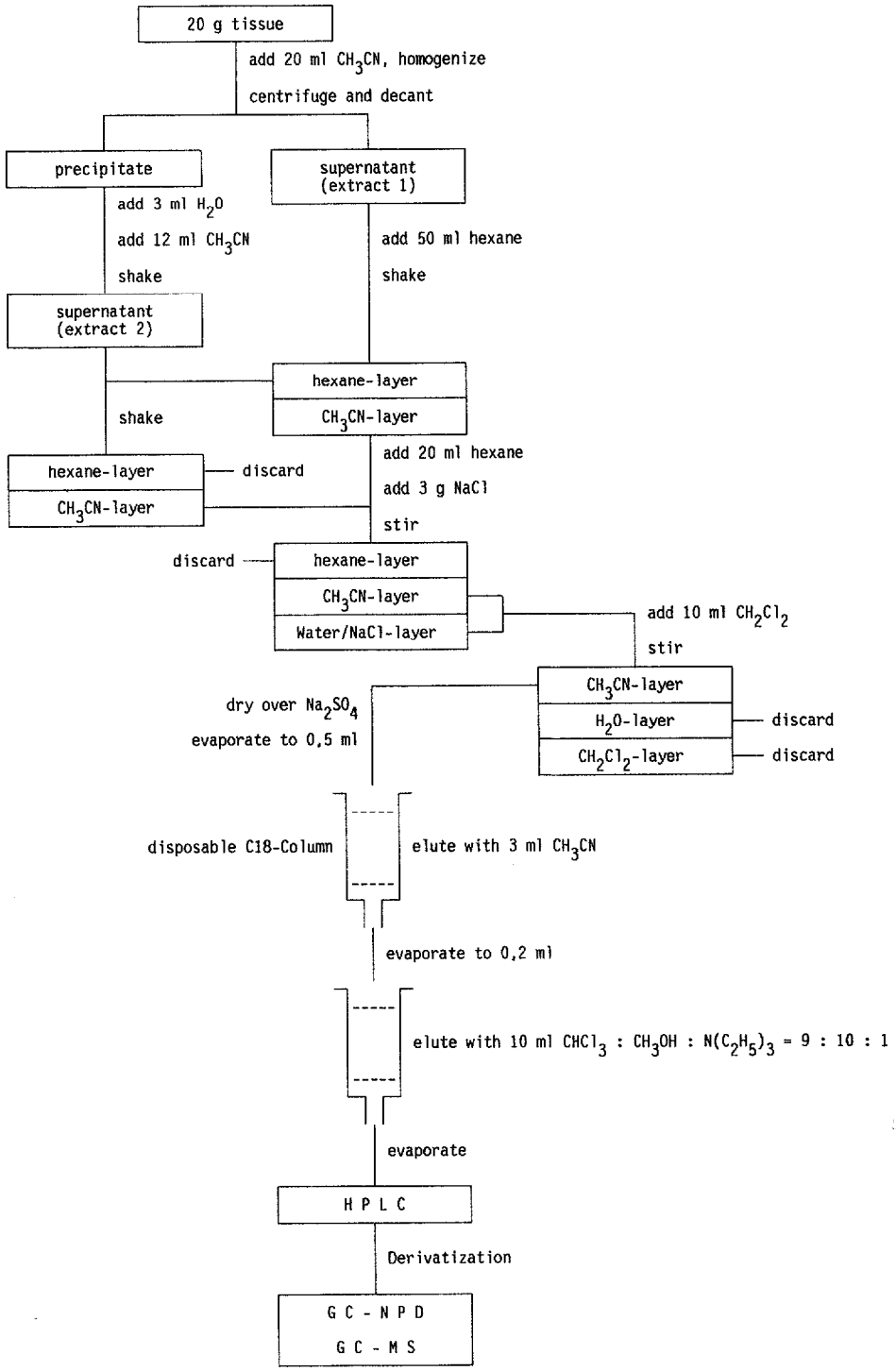


Fig. 2. Extraction procedure.

HPLC. After the final cleaning step, the eluate is evaporated to dryness in a conical vial under a stream of nitrogen and submitted to the derivatization reaction.

As an alternative, the HPLC sample solution was evaporated in the same way and the vial dried *in vacuo* (20 mbar) over phosphorus pentaoxide at 40°C in an oven (TO-50; Büchi) for 8 h. The residue was dissolved in 2 ml of acetone and filtered through a microfiltration apparatus (13 mm diameter HVHP microfilters, 0.45-μm pore size; Swinny Filter Holder, Millipore). The solution was then submitted to derivatization.

Derivatization procedures. To prepare methyl derivatives, the residue was dissolved in 2 ml of acetone and 30 μl of an aqueous 30% (m/v) sodium carbonate solution and 50 μl of a 10% (v/v) solution of methyl iodide in acetone were added. The mixture was shaken on a Vortex mixer, the reaction vial was well sealed and the mixture was heated in a paraffin bath at 60°C for 30 min. After cooling, the mixture was evaporated to dryness with a gentle stream of nitrogen. To the residue, 1 ml of ethyl acetate and 1 ml of water were added, the mixture was shaken on a Vortex mixer and the ethyl acetate phase was removed. The extraction was repeated with two portions of 1 ml of ethyl acetate. The combined ethyl acetate solutions were evaporated to 0.1 ml by a stream of nitrogen after drying over sodium sulphate. A 1-μl volume of this solution was injected into the GC system.

For pentafluorobenzyl derivatives, the procedure was the same as for the methyl derivatives except that 50 μl of a 1% (v/v) pentafluorobenzyl bromide solution were used instead of the methyl iodide solution.

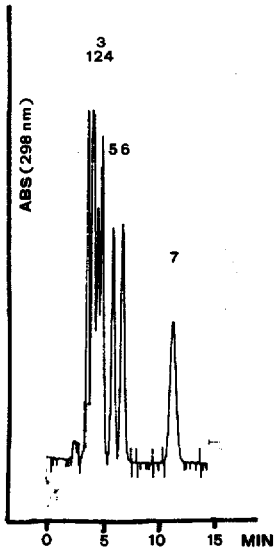
Chromatography. A 1-μl volume of the sample solution was injected in the splitless mode. The needle of the syringe was a long type (7 cm). The temperature programme is described under *Instrumentation*.

RESULTS AND DISCUSSION

Detection by HPLC

For the detection of the benzimidazoles in tissues containing large amounts of interfering substances such as liver or kidney, a laborious clean-up was necessary; the use of acetonitrile for the extraction resulted in a recovery of better than 50% in all instances. Acidic extraction procedures did not result in better recoveries. The extraction and defatting step caused a loss of a few percent of the substances. The high content of matrix substances in the resulting sample solution required the application of further clean-up steps such as chromatography on disposable reversed-phase and Florisil cartridges. Experiments with preparative thin-layer chromatography resulted in a severe loss of sample substance and an increase in interferences in HPLC caused by the extraction of plate-derived substances. For muscle tissues the chromatographic clean-up on the Florisil cartridge may be omitted. It did not seem that the benzimidazoles form conjugates in large amounts; recovery studies were performed on tissue samples from a pig administered a therapeutic dose of fenbendazole (**2**). The samples were treated with β-glucuronidase in conjunction with arylsulphatase (Glusulase; Boehringer, Mannheim, F.R.G.) or by simple hydrolysis with acids and the contents of treated and untreated samples were determined by HPLC; the results showed no difference.

The HPLC separation with the ion-pair reagent pentasulphonate at pH 3.5 was



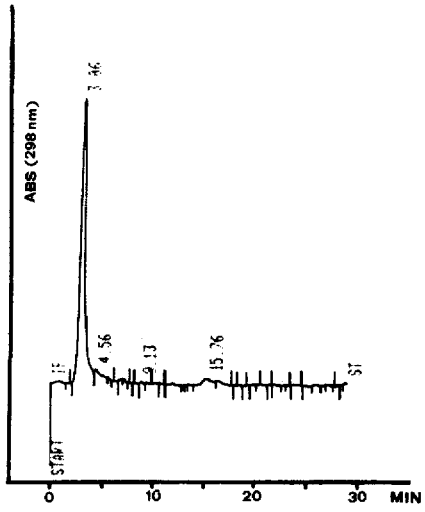


Fig. 3. Chromatogram of standard solution of (1) oxfendazole, (2) thiabendazole, (3) mebendazole, (4) flubendazole/oxibendazole, (5) albendazole, (6) fenbendazole and (7) triclabendazole obtained using mobile phase 1. Injected amounts: 100 ng of each substance in 10 μl. Absorbance range setting: 0.5 a.u.f.s.

Fig. 4. Chromatogram of typical extract of an unfortified muscle of a pig obtained using mobile phase 1. Injection volume: 10 μl (sample solution: 500 μl, corresponding to 10 g of sample). Absorbance range setting: 0.05 a.u.f.s.

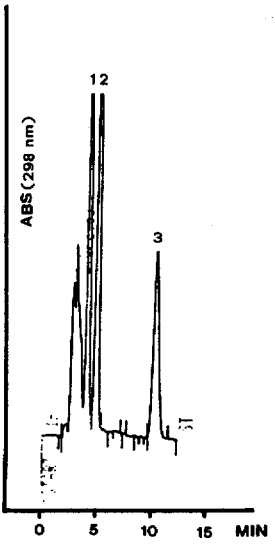


Fig. 5. Chromatogram of typical extract of muscle of a pig after administration of fenbendazole obtained using mobile phase 2. Peaks: (1) fenbendazole (0.5 mg/kg); (2) sulphone metabolite (1 mg/kg); (3) oxfendazole, another metabolite of fenbendazole (1.4 mg/kg). Injection volume: 10 μl (sample solution: 500 μl, corresponding to 10 g of sample). Absorbance range setting: 0.05 a.u.f.s.

TABLE II

TYPICAL RETENTION TIMES ON AN RP-18 COLUMN

Benzimidazole	*Retention time (min)*		λ_{max} *(nm)*[a]	ε *(l mol^{-1} cm^{-1})*[a]
	Mobile phase 1	*Mobile phase 2*		
Oxfendazole (**5**)[b]	3.7	3.9	295	18 200
Thiabendazole (**7**)	4.2	4.6	301	24 200
Mebendazole (**4**)	4.8	5.8	314	14 100
Flubendazole (**3**)	4.9	6.2	312	14 600
Oxibendazole (**6**)	5.1	6.3	297	13 500
Albendazole (**1**)	6.3	8.5	297	12 400
Fenbendazole (**2**)	7.2	10.4	297	14 700
Triclabendazole (**8**)	12.4	20.0	306	19 100

[a] Measured in methanol.
[b] In mobile phase 3: 4.8 min.

the most suitable for the detection of all eight benzimidazoles in one step; ion-suppression systems resulted in longer retention times, poorer separations and considerable tailing of the peaks. The mobile phases described allow the benzimidazoles to be identified and determined in tissue samples, but a baseline separation of the eight benzimidazoles was not achieved. Mobile phase 1 was used for routine analysis and screening. For the verification of oxfendazole (**5**) in liver and kidney samples, mobile phase 2 was used; a better separation from interfering matrix peaks was not possible. Mobile phase 3 was used for the quantification of oxfendazole (**5**) in liver and kidney.

UV detection of the benzimidazoles at 298 nm was used, and the UV spectra show two maxima at about 250 and 300 nm (see Table II). The more intense maxima at 250 nm were not suitable for detection because of self-absorption of the mobile phases. In order to improve the detection limit, fluorescence emission could be used, but only for albendazole (**1**), oxibendazole (**6**) and thiabendazole (**7**) was a gain in sensitivity in

TABLE III

TYPICAL RECOVERIES FOR A CONCENTRATION RANGE OF 0.1 mg/kg

Contents in spiked samples of 10 g each determined by HPLC.

Benzimidazole	*Recovery (%)*[a]		
	Liver	*Kidney*	*Muscle*
Albendazole (**1**)	71 (4.1)	74 (3.9)	71 (5.7)
Fenbendazole (**2**)	87 (3.6)	86 (4.2)	77 (3.5)
Flubendazole (**3**)	70 (7.8)	74 (2.8)	73 (5.8)
Mebendazole (**4**)	65 (5.1)	72 (3.8)	66 (11.6)
Oxfendazole (**5**)	45 (7.6)	39 (4.4)	80 (6.6)
Oxibendazole (**6**)	75 (6.9)	79 (4.0)	70 (8.2)
Thiabendazole (**7**)	66 (4.4)	66 (2.2)	66 (5.5)
Triclabendazole (**8**)	74 (5.3)	71 (2.9)	72 (2.8)

[a] Relative standard deviations (%) (n = 4) in parentheses.

relation to UV detection obtained; unequal excitation and emission wavelengths rendered the test suitable for only one substance in one chromatographic run.

In the region of 0.1 mg/kg, the recoveries obtained were 66–87% with a relative standard deviation of 3–11%. High relative standard deviations were obtained for liver samples containing large amounts of interfering substances (see Table III).

The detection limits were in the range 20–50 μg/kg, depending on the matrix (for the standard solutions, 1–4 ng per injection, determined on the basis of a signal-to-noise ratio of 1:3). The quantification was accomplished by integration of the peak areas and using external standards. If the detection involved all eight benzimidazoles, an internal standard could not be used. Linear regression was determined within the range 10–200 ng. For typical chromatograms see Figs. 3–5.

Detection by GC and GC–MS

As the benzimidazoles are basic and exhibit low volatility, a derivatization procedure was inevitable. Only thiabendazole (**7**) and triclabendazole (**8**) are accessible by direct GC, although with a high detection limit.

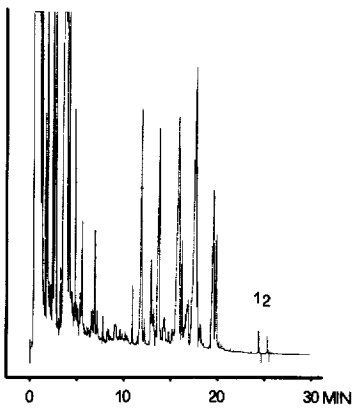
Of the derivatization reactions generally used in the GC determination of substances with amino functions, we chose acylation with trifluoroacetic anhydride, heptafluorobutyric anhydride, N-methyltrifluoroacetamide and pentafluorobenzoyl chloride and alkylation with methyl iodide and pentafluorobenzyl bromide.

Nose *et al.*[10] described the acylation of thiabendazole with pentafluorobenzoyl chloride for GC with electron-capture detection (ECD). We tried the same derivatization with the present benzimidazoles, but only for thiabendazole (**7**) was a derivative detectable; triclabendazole (**8**) and the benzimidazoles of the carbamate type (**1–6**) yielded no results. Although in HPLC and thin-layer chromatography reaction products could be recognized, the derivatives of these substances decomposed during GC. Cline *et al.*[14] and Tjan and Jansen[13] obtained similar results with chemically related carbamate pesticides.

For this reason, we applied the Claisens carbonate alkylation method[16] using the reagents cited above for all eight benzimidazoles. Tjan and Jansen[13] described a similar procedure for the determination of thiabendazole (**7**) and carbamate fungicides in fruits.

Although the detection of the pentafluorobenzyl derivatives by ECD could be very sensitive, we used thermionic nitrogen–phosphorus detection (NPD), as it is less affected by impurities in the matrices. As shown by GC–MS (see below), for the benzimidazoles of the carbamate type (**1–6**) the N,N′-dialkyl derivatives were always obtained. If two tautomeric forms are possible, the derivatives always appeared as two well separated peaks. Figs. 6–8 show typical chromatograms for liver, kidney, tissue and blood samples. On a non-polar capillary column, *e.g.*, of the OV 1-type, the alkyl derivatives normally appear in the following order: thiabendazole (**7**), oxibendazole (**6**), albendazole (**1**), triclabendazole (**8**), fenbendazole (**2**), flubendazole (**3**), mebendazole (**4**) and oxfendazole (**5**). The peaks of these derivatives appeared in a region of the chromatogram in which no interference from matrix peaks occurred.

Verification was accomplished by comparison with external standards on two or more chromatographic systems. As the derivatives undergo decomposition during the injection and chromatography owing to the high temperatures needed, quantitative determinations were not possible by GC.



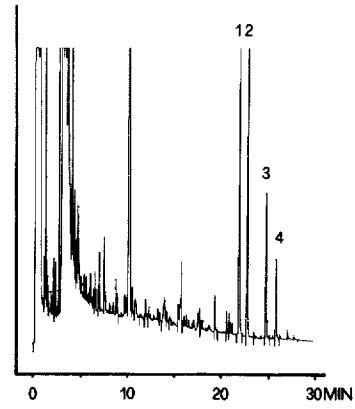
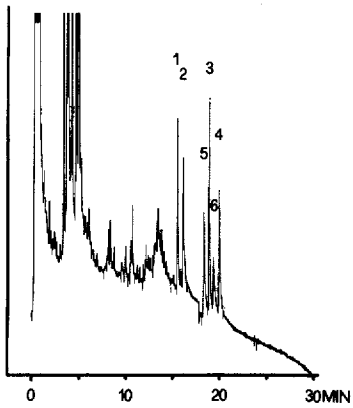


Fig. 6. Typical chromatogram of a liver sample spiked with 0.1 mg/kg mebendazole, obtained with NPD. Peaks 1 and 2 are pentafluorobenzyl derivatives.

Fig. 7. Typical chromatogram of a kidney sample from a pig administered with fenbendazole, obtained with NPD. Peaks: 1 and 2, pentafluorobenzyl derivatives of fenbendazole; 3 and 4, pentafluorobenzyl derivatives of the sulphone metabolite of fenbendazole.

The derivatives were more useful for verification by GC–MS. With the methyl derivatives, the electron-impact (EI) mass spectra of each of the derivatives exhibit a series of a few ions. Generally, the molecular ion appears as the base peak. Then a characteristic fragmentation of the benzimidazoles of the carbamate type (**1–6**) is the loss of the neutral mass 59, corresponding to the carbamic acid methyl ester moiety, the resulting fragment appearing as an abundant ion, often as the base peak. The derivative of triclabendazole (**8**) shows the typical isotopic composition of four molecular ions corresponding to the isotopic combinations of three chlorine atoms.



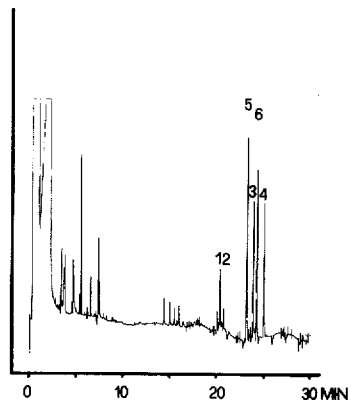


Fig. 8. Chromatograms of muscle tissue (left) and blood (right) from a pig administered fenbendazole, obtained using NPD. Methyl derivatives: 1, 2, of fenbendazole; 3, 4, of a sulphone metabolite; 5, 6, of oxfendazole, also appearing as a metabolite.

TABLE IV

ELECTRON-IMPACT (EI) AND POSITIVE-ION (PICI) AND NEGATIVE-ION (NICI) CHEMICAL-IONIZATION MASS SPECTRA [m/z VALUES (RELATIVE INTENSITIES)]

Benzimidazole	*Methyl derivatives: EI spectra*	*Pentafluorobenzyl derivatives*		
		EI spectra	*PICI spectra*	*NICI spectra*
Albendazole	293 (100%) M^+ 234 (32%) 192 (45%) 72 (19%) 59 (23%)	625 (100%) M^+ 566 (25%) 444 (15%) 181 (86%)	654 (13%) 626 (100%) 446 (32%) 182 (75%) 181 (77%)	444 (100%)
Fenbendazole	327 (100%) M^+ 268 (91%) 59 (38%)	659 (100%) M^+ 600 (27%) 478 (36%) 181 (63%) 59 (33%)	688 (13%) 660 (100%) 480 (18%) 182 (24%) 181 (17%)	478 (100%)
Flubendazole	341 (65%) M^+ 282 (100%)	673 (22%) M^+ 614 (23%) 181 (90%) 123 (100%) 59 (30%)	702 (13%) 674 (100%) 494 (19%) 182 (44%) 181 (50%)	492 (100%)
Mebendazole	323 (63%) M^+ 264 (100%) 246 (23%) 159 (23%) 105 (44%) 77 (76%) 59 (27%)	655 (34%) M^+ 596 (30%) 181 (79%) 105 (100%) 77 (81%) 59 (26%)	684 (15%) 656 (100%) 476 (16%) 182 (64%) 181 (64%)	474 (100%)
Oxfendazole	343 (57%) M^+ 327 (37%) 295 (64%) 266 (75%) 250 (30%) 236 (100%) 159 (73%) 72 (76%) 59 (79%)	675 (28%) M^+ 659 (16%) 627 (4%) 600 (6%) 181 (100%) 59 (43%)	704 (7%) 676 (100%) 660 (17%) 85 (25%) 73 (20%) 57 (65%)	494 (100%) 478 (82%)
Oxibendazole	277 (100%) M^+ 235 (27%) 234 (31%) 218 (27%) 176 (98%)	609 (79%) M^+ 550 (24%) 508 (11%) 428 (17%) 386 (35%) 181 (100%) 59 (23%)	638 (16%) 610 (100%) 568 (8%) 430 (9%) 182 (14%) 181 (11%)	428 (100%)
Thiabendazole	215 (100%) M^+ 187 (31%) 156 (23%) 155 (24%) 77 (41%)	381 (100%) M^+ 362 (65%) 303 (27%) 214 (43%) 181 (54%) 90 (66%)	410 (24%) 382 (100%) 202 (46%) 182 (38%) 181 (27%)	200 (100%)
Triclabendazole	372/374/376/378 M^+ (75/67/24/8%) 339/341/343/345 (100/67/24/6%)	538/540/542/544 M^+ (45/42/14/3%) 357/359/361/363 (42/58/28/2%) 181 (100%)	567/569/571/573 (8/9/3/1%) 539/541/543/545 (97/100/59/7%) 359/361/363/365 (19/17/6/2%) 182 (21%) 181 (17%)	357/359/361/363 (100/98/37/5%)

The loss of the neutral mass of 33 (probably HS) leads to an analogous isotopic pattern of prominent peaks, including the base peak.

The relatively simple spectra of these derivatives with their few, abundant heavy fragments are suitable for EI–MS in the scan- or fragmentation mode (MID, SIM).

Although the pentafluorobenzyl derivatives are chromatographically less suitable than the methyl derivatives, because the molecular mass often reaches high values, they are very useful in detection by chemical ionization (CI) MS techniques, *e.g.*, for pulsed positive ion–negative ion chemical ionization.

In EI–MS, the benzimidazoles of the carbamate type (**1–6**) produce a set of prominent ions, including the molecular ion (most abundant), the fragment of *m/z* 181, corresponding to the pentafluorotropylium ion, a fragment formed by the loss from the molecular ion of neutral mass 59, which is similar to the fragmentation pattern of the methyl derivatives, and a less abundant ion arising from the loss of the pentafluorobenzyl moiety, $[M - 181]^+$. The fragment of *m/z* 181 often appears as the base peak, a fact that renders these EI spectra unsuitable for the fragmentation MS (MID, SIM), the ion of *m/z* 181 also being ubiquitous in the matrix spectra.

The positive-ion CI (PICI) spectra show as a common feature the quasimolecular ion $[M + 1]^+$ as the base peak and an ion $[M + 29]^+$, typical of PICI using methane as the reactant gas. The pentafluorotropylium ion (*m/z* 181) and the protonated analogue (*m/z* 182) are also of important abundance. The derivative of triclabendazole (**8**) shows the characteristic isotopic profiles for the quasimolecular ion $[M + 1]^+$, the ion $[M + 29]^+$ and the probably protonated fragment resulting from the loss of the pentafluorobenzyl moiety, $[M - 180]^+$.

The negative-ion CI (NICI) spectra result in a single abundant fragment $[M - 181]^-$. This fragmentation has its counterpart in the removal of the pentafluorobenzyl moiety in the PICI spectra; the fragmentation seems to follow an ion-pair mechanism.

The EI- and CI-MS results are summarized in Table IV.

Practical application

In order to verify that the methods do indeed work, a pig was fed 15 mg of fenbendazole (**2**) per kg body weight in the feed, and was slaughtered 24 h after the application. Fenbendazole and two metabolites were found in the tissues (Table V,

TABLE V

RESIDUES IN THE TISSUES OF A TREATED PIG

Benzimidazole	*Concentration (ppm)*[a]		
	Liver (n = 3)	*Kidney (n = 3)*	*Muscle*[b] *(n = 9)*
Fenbendazole (**2**)	12.50 (2.9)	2.83 (4.4)	0.44 (7.5)
Oxfendazole (**5**)	0.17 (30.6)	n.d.[c]	1.34 (8.5)
Sulphoxide metabolite	4.12 (1.5)	2.00 (5.2)	1.14 (7.1)

[a] Relative standard deviations (%) in parentheses.
[b] Samples were taken from different muscles.
[c] Not detected.

Fig. 5). The metabolites were identified as the sulphoxide metabolite, which corresponds to oxfendazole (**5**), and the sulphone metabolite.

To check the practical routine application of the methods, the tissues (muscle, kidney, liver) from 43 normal slaughtered pigs and 25 forced slaughtered cattle were examined. No residues of benzimidazoles were found. The analysis showed that the described method is routinely practicable.

CONCLUSION

The method described here is suitable for detecting and verifying the benzimidazole anthelmintics albendazole, fenbendazole, flubendazole, mebendazole, oxfendazole, oxibendazole, thiabendazole and triclabendazole in tissue samples with a detection limit of 20–50 μg/kg. The verification of the results is performed by GC–MS or GC–NPD methods using the methyl or pentafluorobenzyl derivatives of the drugs.

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