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Determination of benzimidazole residues using liquid chromatography and tandem mass spectrometry

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Abstract

The determination of residues of benzimidazole using liquid chromatography and tandem mass spectrometry (LC–MS–MS) with ion spray ionization is described. Swine muscle tissue was spiked with a mixture of fifteen benzimidazoles, including metabolites of fenbendazole and albendazole. As clean-up procedure, an ethyl acetate extraction followed by solid-phase extraction on styrol-divinyl-benzene cartridge was used. The evaluation was performed by selecting the characteristic product ions for the benzimidazoles and using multiple reaction mode. 2-*n*-Butylmercaptobenzimidazole was used as internal standard. Blank muscle samples were fortified in the concentration range of 1–22 μg/kg. The limits of detection were below 6 μg/kg and the limits of quantification for most benzimidazoles were below 10 μg/kg. The matrix effect was checked using spiked muscle tissues of cattle and sheep as well as liver of cattle. Practical application will be shown by incurred egg material from laying hens treated with flubendazole. The recovery of the clean-up was mostly above 50% in muscle tissue and 70% in egg yolk.

Keywords: Benzimidazole

1. Introduction

Benzimidazoles are substances with antiparasitic effects (part of the group of the anthelmintics). They are widely used in veterinary medicine against endoparasites in food producing animals. EU-Regulation requires an analytical procedure for the control of the maximum residue limits (MRL). This MRL is defined, for example, for fenbendazole as the sum of the extractable residues that may be oxidized to oxfendazole sulfone (that amounts to 50 μg/kg in muscle, fat and kidney and 1000 μg/kg in the liver). For albendazole and albendazole sulfoxide a provisional MRL of 100 μg/kg in muscle tissue was published [1–3]. The MRL for flubendazole is 400 μg/kg in egg [4].

For the determination of benzimidazole residues HPLC with UV detection was frequently used [5–15]. The use of HPLC with fluorescence detection was reported as well [16]. The combination of HPLC with thermospray MS for the determination of fenbendazole and its metabolites in muscle tissue has been considered by Blanchflower et al. [17]. Gas chromatography with electron capture detection after benzoylation was the detection method used by Nose et al. and Bardalaye et al. [18,19]. Furthermore, thiabendazole residues were detected by gas chromatography after benzylation [18] and methylation [20,21]. Lafuente and coworkers and Oishi et al. [22,23] reported about the determination of thiabendazole without derivatization using the nitrogen–phosphorus GC-detector. Amijee and Wells [24]

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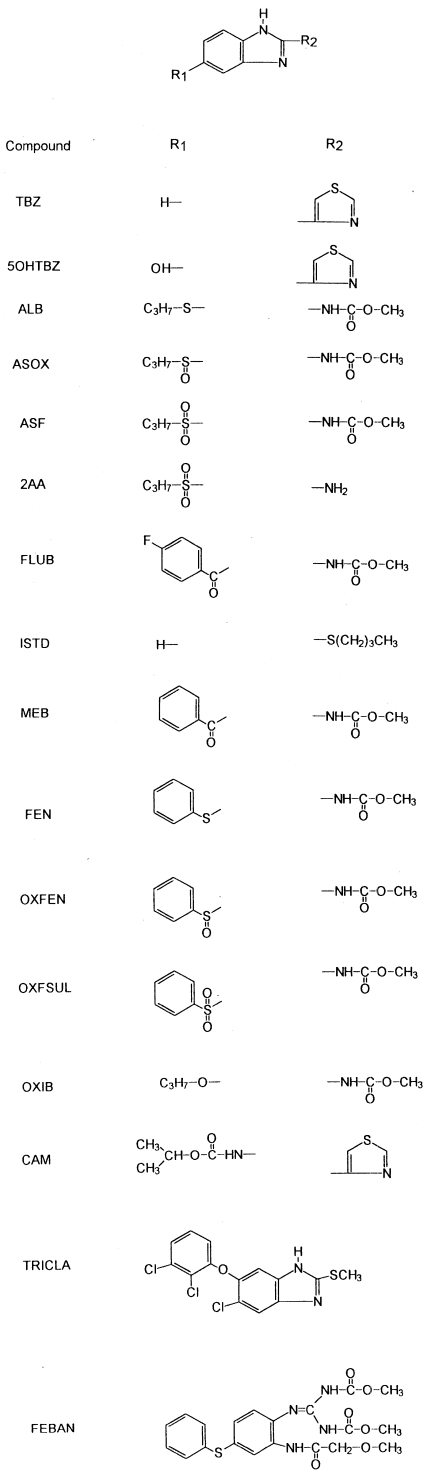

Fig. 1. Structure of the benzimidazoles.

developed a method for the determination of fenbendazole residues with on-column methylation. For confirmatory analysis several methods, based on gas chromatography and mass spectrometry, after silylation [5], methylation [15] and benzylation [15] were published. Allan and Watson identified the biliary metabolites of mebendazole by mass spectrometry [25]. Gas chromatography with high resolution mass spectrometry for the identification of thiabendazole, albendazole and fenbendazole residues in muscle tissue was reported as well [26,27]. However, methods based on gas chromatography are not suitable for quantification due to the thermal decomposition in the gas chromatograph or due to non-reproducible ionisation in the source of the mass spectrometer.

Therefore, the aim of this paper is to describe a confirmatory method based on mass-related data which is suitable for quantitative determination of benzimidazole residues in muscle tissue in a concentration range corresponding to MRL-Regulations. The molecular structure of the antiparasitics studied in this work are shown in Fig. 1.

2. Experimental

2.1. Standards and chemicals

All solvents were residue-analysis grade unless stated otherwise. Ethanol 99.5% LiChrosolv was used. Demineralized water was used in all experiments (Milli-Q Gradient, Millipore).

Thiabendazole was purchased from Aldrich, (No. 28951-5). Other benzimidazole analytical standards were kindly provided by the manufacturers: fenbendazole from Hoechst (Wiesbaden, Germany), mebendazole from Janssen and 2-amino-albendazole-sulfone from Janssen Pharmaceutica (Beerse, Belgium), 5-hydroxy-thiabendazole and cambendazole from MSD Sharp & Dohme (Rohrdorf, Germany), febantel from Bayer AG (Leverkusen, Germany), oxfendazole, albendazole, 2-amino-albendazole-sulfone and albendazole sulfone from SmithKline Beecham (Louvain-la-Neuve, Belgium), albendazole sulfoxide from Formil Chemica (Sao Paulo, Brazil), oxfendazole sulfone from Hoechst Roussel Vet GmbH (Wiesbaden, Germany), triclabendazole from Ciba-Geigy GmbH (Frankfurt a.M. Germany), oxibendazole from Vetimex B.V. (Bladel, The Nether-

lands) and flubendazole from AniMedica (Münster, Germany). 2-*n*-Butyl-mercaptobenzimidazole as internal standard was synthestized as proposed by Saxena and Knobloch [28,29]. Styrol-divinyl-benzene (SDB) cartridges were purchased from Baker (No. 7519-05). Ethanol–0.2 N HCl solution was prepared by mixing of 66 ml ethanol with 33 ml 0.2 N HCl. Poly(propylene glycole) for the calibration of MS was purchased from SCIEX (No. 401936).

2.2. Standard solutions

A stock solution containing 1 mg/ml of benzimidazole in dimethylsulfoxide, working standard solution containing 10 μg/ml in methanol were prepared. Before analysing the standard solutions, the working standard solution was evaporated to dryness and then dissolved in 1 ml of the HPLC mobile phase.

2.3. Instrumentation

The LC–MS–MS system comprised a series 200 micro LC-pump with series 200 autosampler and a vacuum degasser, coupled with an API 365 tandem mass spectrometer with ion spray interface (SCIEX, Division of Perkin-Elmer, Überlingen, Germany). A vacuum centrifuge (UniEquip, Martinsried, Germany) was used for evaporation of the solvents. The LC-column was a Zorbax RX C_{18} 2.1×15 cm, 5 μm (ASS GmbH, Germany), the photodiode array detector was a W 990 (Waters, Eschborn, Germany).

2.4. Sample preparation – muscle and liver samples

The clean-up procedure was based on the method of Wilson et al. [5].

Muscle and liver samples (3 g) were weighed into 50 ml centrifuge tubes. Internal standard, if necessary stock solutions, 1.5 g sodium sulfate, 0.5 ml 4 *M* potassium carbonate and 5 ml ethyl acetate were added to each sample and mixed using a vortex mixer (30 s). After centrifugation (5 min, 2500 *g*) the organic phase was separated. This extraction procedure was repeated once. The collected organic phases were evaporated to dryness under nitrogen (50°C) or in a vacuum centrifuge. A 5 ml amount of *n*-hexane was given to the dried residue and shaken to get a good mixture. A 1 ml amount of ethanol–0.2 N HCl solution was added to each tube and mixed on a vortex. After centrifugation (2 min, 1000 *g*) the upper layer was discarded. The ethanol–acid layer was evaporated in the vacuum concentrator and then dissolved in 0.5 ml 0.01 *M* ammonium acetate (pH= 5.5) and 0.5 ml methanol. For solid-phase extraction the solution was applied onto the SDB column conditioned with 3 ml methanol and 3 ml distilled water. The column was washed with 3 ml H_2O and 2×3 ml H_2O–methanol (1:1) and was dried under vacuum. After elution with 3 ml methanol–ethyl acetate (1:4) the extract was dried in the vacuum concentrator and the residue was dissolved in 250 μl of the HPLC mobile phase.

2.5. Sample preparation – egg samples

A 3 g amount of egg white or yolk were homogenized in a 50 ml centrifuge tube. Internal standard, if necessary stock solutions, 1.5 g sodium sulfate, 0.5 ml 4 *M* potassium carbonate and 5 ml ethyl acetate were added to each sample and mixed using a vortex mixer (10 s) and ultrasonic bath (15 min). After centrifugation (5 min, 2500 *g*) the organic phase was separated. This extraction procedure has been repeated once. The following steps are identical with those of the clean-up for muscle tissue.

2.6. Biological samples

Flubendazole-free eggs were collected from laying hens grown on a farm owned and maintained by the Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin. Naturally contaminated egg material was obtained by treating 28 hens (Isar-Worsen-Brown crossbred with Araucane) with the veterinary drug ‘Flubendazole 5%’ powder (Bioptivet, Germany) administered for one day via the drinking water. The eggs were collected over a period of 12 days.

2.7. Instrumental conditions

2.7.1. HPLC

The mobile phase – acetonitrile (60% v/v), 0.01 *M* ammonium acetate and 0.5% acetic acid (40%) – was pumped with a flow-rate of 40 μl/min for ion spray and 200 μl/min for turbo ion spray. The

injection volume was 4 μl for ion spray and 15 μl for turbo ion spray, respectively.

2.7.2. UV detector

Photodiode array detector was used with the following parameters: wavelength from 190 to 380 nm, resolution 2 nm, sampling time 112 ms, accumulation five times, sensitivity 0.03 AUFS.

2.7.3. Mass spectrometer

The ion spray interface was used with positive ion detection. Nitrogen (purity grade 5.0) was used as nebulizer-, collision-, curtain- and heater-gas. The flow-rate of the heater gas for turbo ion spray was 8 l/min.

The calibration was done with poly(propylene glycole). For optimizing the mass spectrometer direct infusion with a flow of 20 μl/min was used.

The parameters for all compounds measured by turbo ion spray were as follows: temperature: 450°C, ion spray voltage: 4600 V, orifice: 40 V, focusing ring: 200–250 V, entrance quadrupole: −3 to −11 V, inter-quadrupole lens 1: −6.3 V, prefilters: −11.3 V, first resolving quadrupole −6.5 V, inter-quadrupole lens 2: −18 to −23 V, collision cell quad: −30 to −65 V, inter-quadrupole lens 3: −50 to −190 V, last resolving quadrupole: −150 V, electron multiplier 1800 V.

The instrument was operated either in full scan mode (Q 1 scan) to collect spectra or in the multiple reaction monitoring mode (MRM) for maximum sensitivity. For the former, a dwell time of 2 ms was used and for the latter, the dwell time was 100 ms/ion for muscle samples and 400 ms/ion for egg tissue. Product ion scans were acquired in mass area, depending on the pseudo molecular peak detected in first quadrupole using multi-channel analysis.

3. Results and discussion

The MS–MS system (source and instrumental parameters) was optimized by direct infusion of a standard solution of each benzimidazole. Positive ion spray ionization showed the maximum of ion abundances and was therefore employed for product ion scans. The common parameters, e.g. ion spray voltage, collision gas pressure, electron multiplier, optimized for all compounds, are given under Section 2.7. The variation of the flow-rate of the mobile phase, of the gas volume and the ion spray voltage showed the main effects to the spray stability. As a second step the lens voltages were optimized. Orifice and focusing ring operating conditions were set high enough to reduce the chemical noise but low enough to avoid fragmentation (orifice 40 V, focusing ring 200−250 V).

3.1. LC–MS

Benzimidazole residues were separated on a reversed-phase column and detected with turbo ion spray. The use of ammonium acetate and acetic acid in the mobile phase helps to produce protonated molecular ions (charged droplets). Furthermore, high proton concentrations were required in the solvent to maintain a low enough pH in the droplets and to avoid statistical fluctuations in buffer ion concentration for the electric field. As the benzimidazoles and their product ions have different relative molecular masses, the use of a gradient LC regime to prevent co-elution was not necessary. So a mobile phase with 60% of organic solvent could be used (a decrease of surface tension due to acetonitrile leads to higher efficiency of ion evaporation relative to water).

Full scan spectra (with quadrupole 1) were obtained by direct infusion of standard solution into the ion spray. In all cases the mass spectra were simple and consisted only of a few fragments of the protonated molecular ion $[M+H]^+$. The relevant $[M+H]^+$ ions monitored are listed in Table 1 as Q 1 mass.

3.2. LC–MS–MS

Before multiple reaction monitoring (MRM) experiments could be performed the collision energy (potential difference between collision cell and entrance quadrupole) had to be optimized for each benzimidazole. However, the optimized values may not be the optimum for all systems. Product ion scan spectra were easily achieved by using 200 ng of each benzimidazole. The spectra had shown low fragmentation: the losses of mass were mainly corresponding to carbamic acid methyl ester moiety.

Table 1
Typical ions of the benzimidazoles used for multiple reaction monitoring (Q 1=ion in quadrupole 1, Q 3=product ion in quadrupole 3)

Compound name	Q 1	Q 3
Thiabendazole (TBZ) (a)	202	131
(b)	202	175
2-*n*-butylmercaptobenzimidazole (ISTD)	207	151
5-hydroxy-thiabendazole (5OHTBZ)	218	191
Albendazole (ALB)	266	233
2-amino-albendazole sulfone (2AA)	240	133
Albendazole sulfoxide (ASOX)	282	208.3
Albendazole sulfone (ASF) (a)	298	266.1
(b)	298	224.1
Mebendazole (MEB)	296	264
Febantel (FEBAN) (a)	447.5	382
(b)	447.5	414
Fenbendazole (FEN)	300	268
Oxfendazole (OXFEN)	316.2	159.1
Oxfendazole sulfone (OXFSUL) (a)	332.1	159
(b)	332.1	300.1
Triclabendazole (TRICLA) (a)	359	343
(b)	359	344
Oxibendazole (OXIB)	250	176.1
Cambendazole (CAM)	303	261
Flubendazole (FLUB)	314	281

Oxfendazole has lost its phenylsulfinyl group as well. An overview of ions used for MRM is given in Table 1 (Q 3 ions). For the analysis of the muscle samples on reversed-phase chromatographic column a turbo ion spray was used with higher flow-rates (0.2 ml/min), the sprayer position was changed and the heater gas was switched on (the heated gas increases the efficiency of the ion evaporation). A typical set of single ion chromatograms for negative muscle spiked with a mixed standard at 10 μg/kg is shown in Fig. 2. The chromatograms were clean and showed no interferences from other compounds.

3.3. 'Cross-talk' effects

To determine 'cross-talk' effects (effect between co-eluting analytes) standard solutions of each benzimidazoles were separately injected in duplicate in two concentrations: 4 ng and 180 ng/15 μl injection volume. Each MS–MS channel was monitored. The results of the 180 ng level are shown in Table 2. The 'cross-talk' effects were observed in about 10% of cases and they were found mostly below 10% of the corresponding standard area. The highest effect was measured at the metabolite of oxfendazole (oxfendazole sulfone). The metabolites fenbendazole and oxfendazole are able to produce the same fragment in quadrupole 3 (m/z 159). Febantel, the precursor of fenbendazole, could lead to such effect as well. Therefore, the MRLs for febantel, fenbendazole and oxfendazole are defined as the sum of the extractable residues that may be oxidized to oxfendazole sulfone, i.e. the residues detected by LC–MS–MS should be added. However, the 'cross-talk' effect could be reduced in this case from 20 to 1.2% if a second product ion (m/z 300 instead of m/z 159) was chosen for the acquisition in quadrupole 3. In real samples the detection of more than one benzimidazole is improbable: fixed combination of benzimidazoles in veterinary drugs is not known in Germany and it would not be useful either.

3.4. Muscle samples

Standard curves for each analyte were generated by analyzing negative muscle tissue spiked in duplicate with standard solutions of each of the 16 analytes at 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 μg/kg concentration level (including 10 μg/kg ISTD). The quantification of each compound was made by reference to ISTD area. Linear regression analysis of the data was performed and the coefficient of correlation (r^2-values) was mainly over 0.95 (except for albendazole and febantel). From these results, the limits of detection (LOD) and the limits of quantification (LOQ) for each compound were calculated applying the method of Funk et al. [30]. The results are listed in Table 3. The LODs were also determined using representative blank swine muscle tissue ($n=23$): LOD, defined as the amount of benzimidazole giving a signal of three times the standard deviation of the blank signal around the retention time of the standard were ≤ 1 μg/kg in most cases. Due to the high specificity of the MS–MS in some cases no background peaks were detected.

For the determination of recoveries blank swine muscle tissues were spiked with standard solution at the concentration level of 50 μg/kg ($n=7$, injected twice). Recovery data were obtained by comparing the peak height (or area) of samples spiked pre-extraction and spiked into extracts post-extraction.

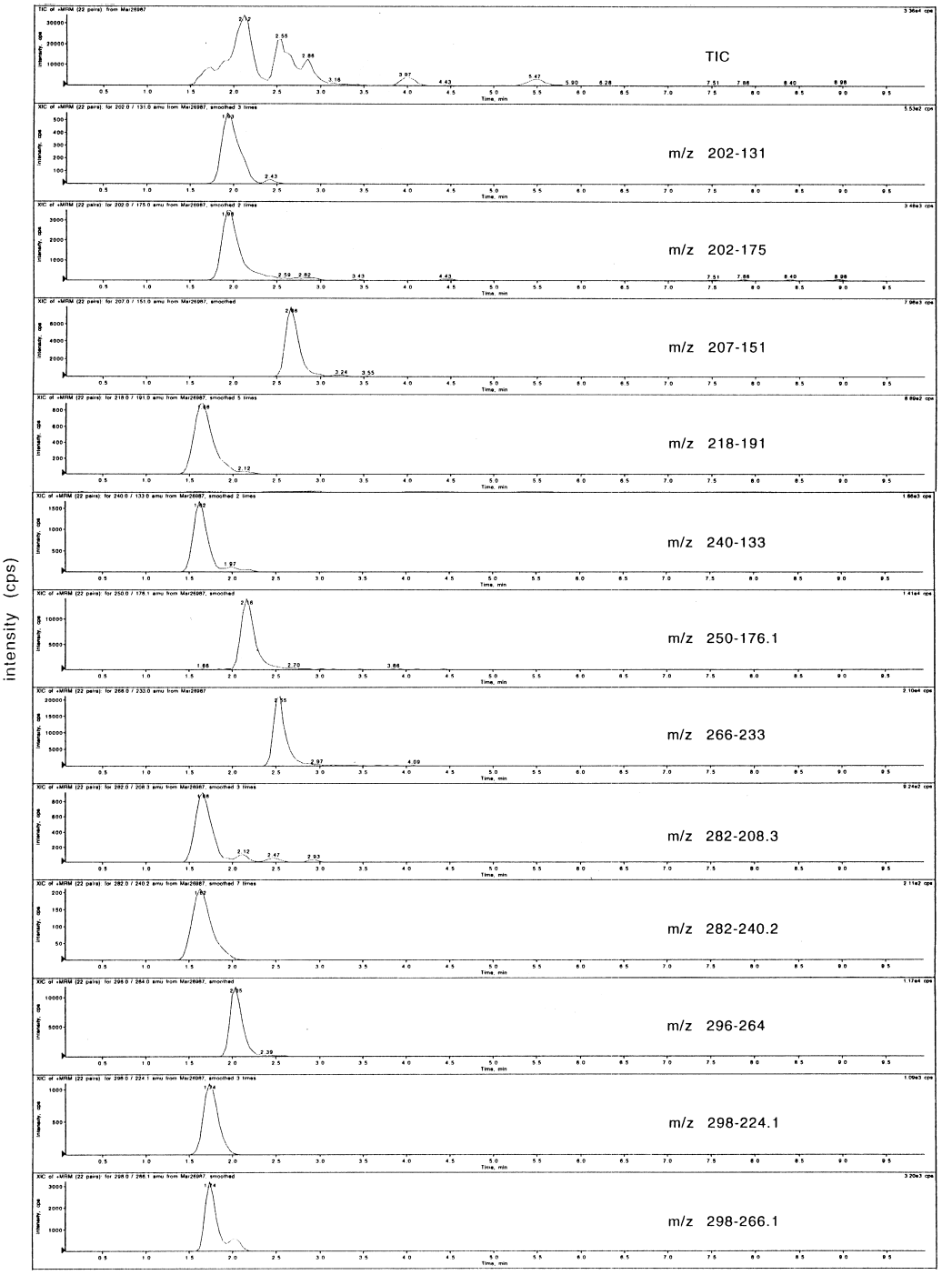

Fig. 2. TIC and ion chromatograms of muscle tissue spiked with 10 μg/kg benzimidazoles and ISTD, acquired by multiple reaction monitoring. Legends to ions are shown in Table 1.

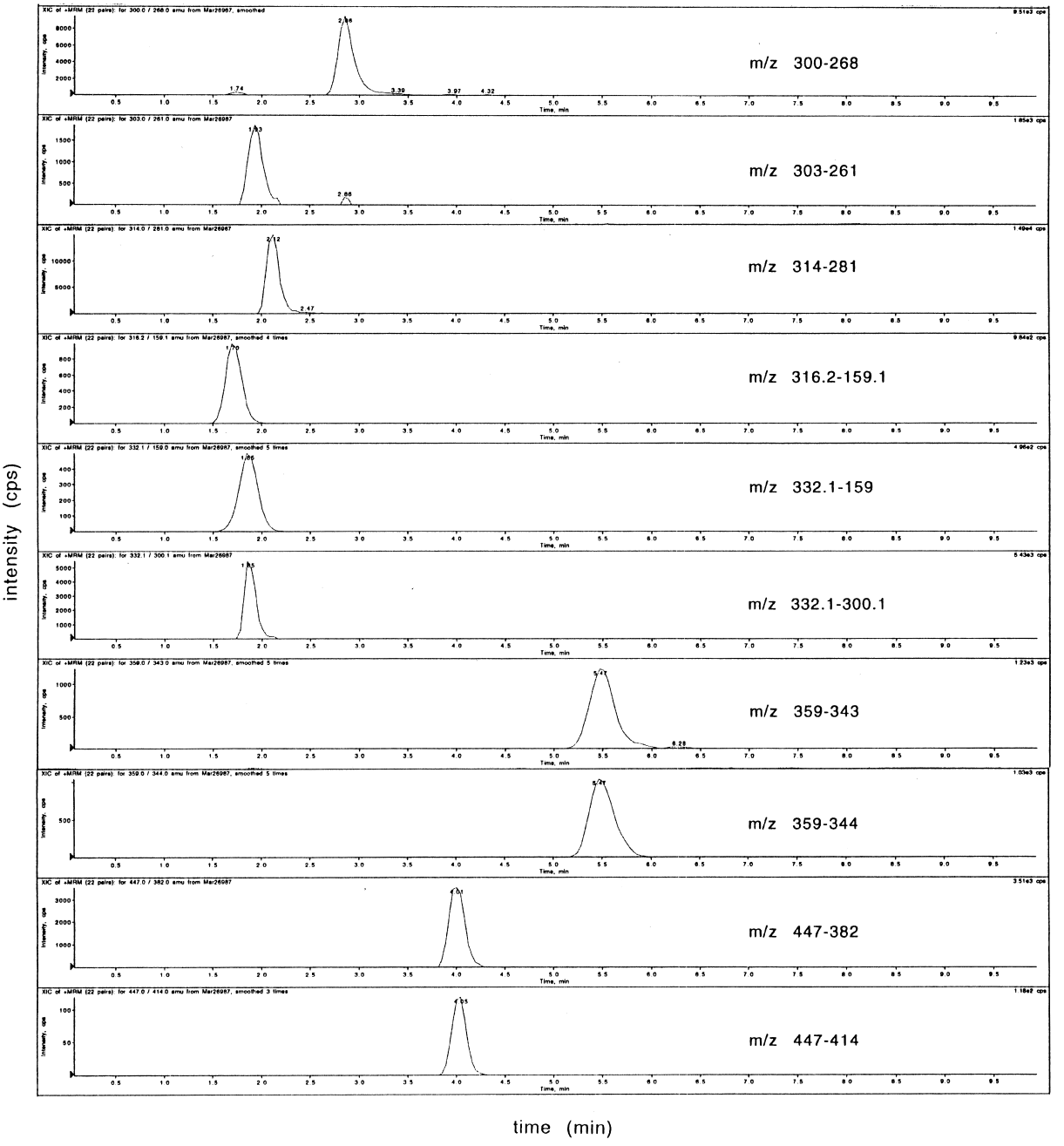

Fig. 2. (*continued*)

The values were mostly over 50%. The results are shown in Table 3. The loss of febantel during the clean-up is extremely high. However, febantel is not suitable as marker residue because it is a prodrug and metabolized to fenbendazole in animals by cyclization. The coefficients of variation (C.V.) of the assay, depending on the substances, were mainly between 8–22%.

Table 2
'Cross-talk' effect in % of area, determined by injection of standard solutions (180 ng/15 μl of each benzimidazole)

Caused by	TBZ	ISTD	5OHTBZ	ALB	2AA	ASOX	ASF	MEB	FEBAN	FEN	OXFEN	OXFSUL	TRICLA	OXIB	CAM	FLUB
TBZ	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ISTD	–	–	–	–	–	0.3	–	–	–	–	0.07	–	–	–	–	–
5OHTBZ	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6	2	–
ALB	–	–	–	–	–	0.5	0.1	–	–	0.2	–	–	–	0.02	–	–
2AA	–	–	–	–	–	7.6	–	–	–	–	–	–	–	–	–	–
ASOX	–	–	–	2.4	0.7	–	–	–	–	–	–	0.4	–	–	–	0.1
ASF	–	–	–	–	–	–	–	9.8	–	0.1	–	–	–	–	–	–
MEB	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FEBAN	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FEN	–	–	–	–	–	–	3.8	–	2.5	–	–	–	–	–	–	–
OXFEN	–	–	–	–	–	–	–	–	–	0.06	–	–	–	–	–	–
OXFSUL	–	–	–	–	–	–	–	–	–	–	20[a]	–	–	–	–	–
TRICLA	–	–	–	–	–	–	–	–	–	–	–	0.35	–	–	–	–
OXIB	–	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–
CAM	–	–	–	–	–	–	0.5	–	–	2.9	–	–	–	–	–	–
FLUB	–	–	–	–	–	0.1	–	–	0.15	–	–	–	–	–	–	–

[a] Determined at m/z 332 to 159, 'cross-talk' effect at m/z 332 to 300 is 1.2%.

3.5. Matrix effect

Photodiode array detector was coupled before MS–MS to evaluate the clean-up selectivity. An aliquot (15 μl) of the tissue extracts was injected onto the HPLC column. In spite of the selective clean-up, co-elution of some endogenous compounds was observed in the front of the chromatogram. This effect was especially pronounced in liver of cattle and less in the muscle tissues.

Therefore, additional standard curves were generated with spiked tissues to exclude 'ion suppression'.

Table 3
Coefficient of correlation (r^2) of the calibration curves, generated by spiked swine muscle tissue in the concentration range of 1–22 μg/kg ($n=17 \ldots 22$)[a,b]

Compound	r^2 Coeff. of corr.	LOD (μg/kg)	LOQ (μg/kg)	Recovery (%)	C.V. (%)
TBZ	0.989	3	5	72	8
ISTD	–	–	–	59	13
5OHTBZ	0.947	7	10	69	12
ALB	0.689	19	30	36	22
2AA	0.976	4	7	47	16
ASOX	0.968	6	9	117	19
ASF	0.985	4	6	81	12
MEB	0.989	3	5	50	15
FEBAN	0.896	10	15	8	28
FEN	0.976	5	7	44	13
OXFEN	0.969	5	8	75	8
OXFSUL	0.980	4	6	54	12
TRICLA	0.973	6	9	45	15
OXIB	0.979	4	6	52	13
CAM	0.981	4	6	49	15
FLUB	0.990	3	5	50	12

[a] Limit of detection (LOD) and limit of quantification (LOD), calculated by the method of Funk et al. [30].
[b] Recovery and the coefficients of variation (C.V.) were determined by analysis of blank muscle samples fortified with 50 μg/kg benzimidazoles ($n=7$).

Table 4
Coefficient of correlation (r^2) of the linear regression (peak area versus concentration) determined with spiked blank tissues in concentration range of 5–40 μg/kg, $n=8\times2$[a,b]

	Muscle of swine	Muscle of sheep	Muscle of cattle	Liver of cattle low conc.	Liver of cattle high conc.
TBZ a	0.978	0.940	0.975	0.978	0.984
TBZ b	0.984	0.967	0.975	0.989	0.983
5OHTBZ	0.946	0.944	0.966	0.971	0.993
ALB	0.955	0.973	0.986	0.980	0.827
2AA	0.957	0.982	0.979	0.952	0.920
ASF a	0.978	0.982	0.986	0.983	0.975
ASF b	0.985	0.982	0.985	0.964	0.971
ASOX	0.960	0.978	0.948	0.940	0.961
MEB	0.990	0.964	0.985	0.974	0.975
FEN	0.989	0.991	0.959	0.974	0.916
OXFEN	0.967	0.982	0.985	0.961	0.977
OXFSUL a	0.972	0.949	0.960	0.943	0.968
OXFSUL b	0.984	0.979	0.975	0.952	0.966
CAM	0.981	0.941	0.979	0.966	0.979
FLUB	0.986	0.980	0.976	0.979	0.958
FEBAN a	0.931	0.980	0.993	0.942	0.495
TRICLA a	0.991	0.989	0.992	0.971	0.937
TRICLA b	0.989	0.993	0.991	0.973	0.923
OXIB	0.982	0.988	0.971	0.982	0.977

[a] Liver of cattle was spiked in two concentration ranges: 5–40 μg/kg ('low conc.') and 100–800 μg/kg ('high conc.').
[b] The letters 'a' and 'b' after the compound name indicate the corresponding ion pairs for multiple reaction monitoring.

Muscle tissues of swine, sheep and cattle, and liver of cattle were spiked in duplicate with standard solutions of each of the 16 analytes at 0, 5, 10, 20 and 40 μg/kg concentration level (including 20 μg/kg ISTD). The MRLs for benzimidazoles in liver tissue reached between 100 and 1000 μg/kg so that liver of cattle was spiked in duplicate with standard solutions of each of the 16 analytes at 0, 100, 200, 400 and 800 μg/kg concentration level (including 400 μg/kg ISTD) as well. Each sample was injected twice.

Linear regression analysis of the peak area versus concentration for each benzimidazoles was performed and the coefficients of correlation (r^2) of calibration curves were calculated. As the results in Table 4 show, the endogenous compounds from different tissues have no significant effect on the quantitation of the method, except for febantel and partially albendazole.

3.6. Egg samples

The limit of detection (LOD) and the limit of quantification (LOQ) for flubendazole were calculated using spiked control egg samples in the concentration range of 50–300 μg/kg ($n=6$) as de-

Table 5
Limit of detection (LOD), limit of quantification (LOD) and the recoveries with the coefficient of variation (C.V.) in egg tissue (yolk), spiked with FLUB and ISTD

Compound egg spiked with (conc.)	LOD (μg/kg)	LOQ (μg/kg)	Recovery (%)	C.V. (%)
ISTD (100 μg/kg)	–	–	77 ($n=7$)	8.1
ISTD (100 μg/kg)			79 ($n=12$)	7.7
ISTD during depletion study (100 μg/kg)			89 ($n=14$)	12.4
FLUB (50–300μg/kg)	32	48	73 ($n=6$)	8.0
FLUB (200 μg/kg)	–	–	70 ($n=12$)	8.0

scribed above. The concentration of the internal standard was 100 μg/kg. Linear regression analysis of the ratio of the area ISTD to FLUB was performed and the r^2-value of calibration curve was 0.998 in yolk. Recoveries were calculated based on results of the following experiments: (a) results of analysis of spiked egg samples at the 50 to 300 μg/kg concentration level, as described above, (b) egg (yolk) were spiked separately with 200 μg/kg FLUB and 100 μg/kg ISTD (n=12) and (c) ISTD areas were compared during the depletion study. The calculated recoveries (in comparison with external standard) in egg yolk were over 70% (contrast to 47% for egg white), The results are shown in Table 5. Within-laboratory reproducibilities were performed by comparison of internal standard area in egg material. Coefficients of variation were below 10% for intra-day and 18% for inter-day precision (n=68).

3.7. Depletion study

A practical application of the assay has been shown with incurred egg material by the concentration-time curves of flubendazole in egg white and yolk. A group of hens (28) were treated with flubendazole. The eggs were collected every day (without relation to individual animals), egg white and yolk were separated and prepared for the analysis. The flubendazole clearance from eggs differs for the white and the yolk. The flubendazole concentration in egg yolk was about five times higher than in the white, therefore this material was chosen for further investigations. The depletion graphs of

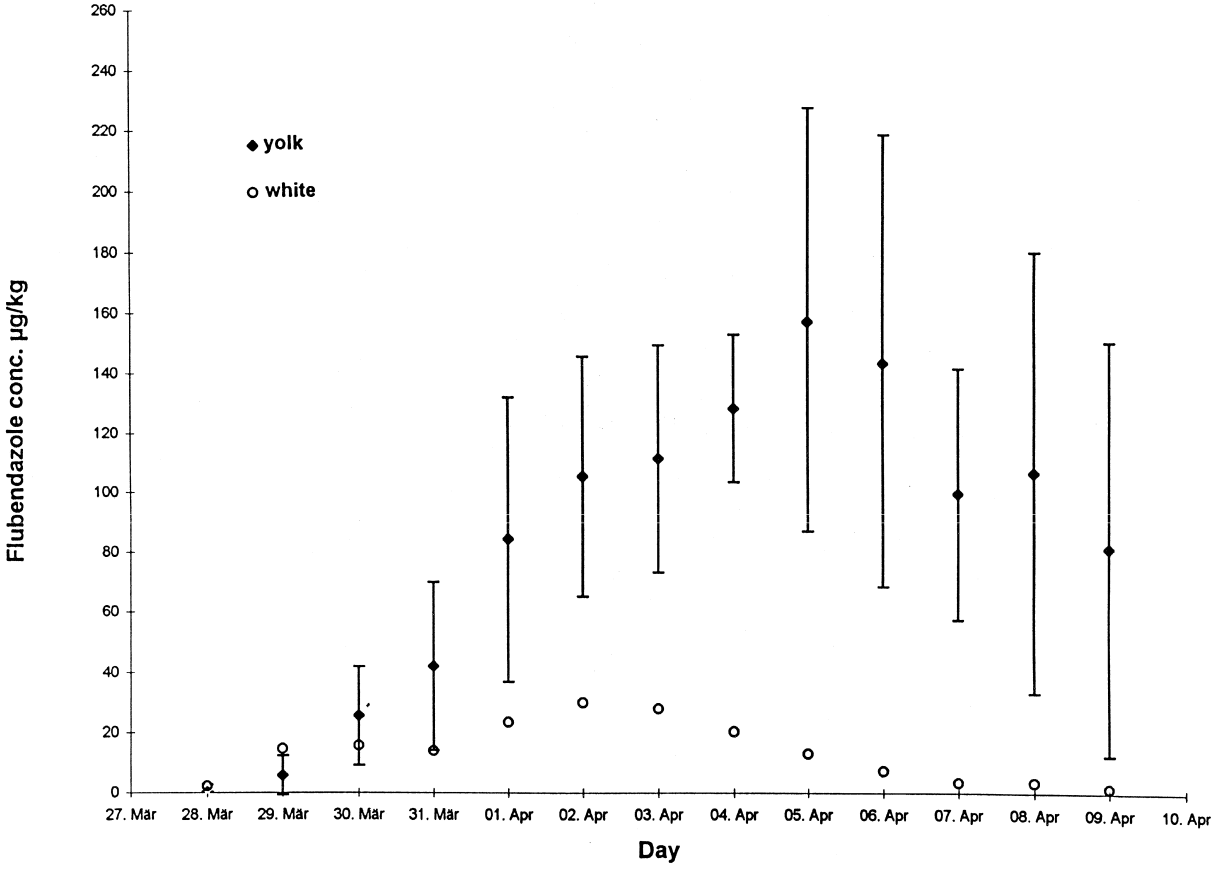

Fig. 3. Concentration-time curves of flubendazole in incurred egg white and yolk. Mean values with standard deviation show the individual pharmakokinetic.

flubendazole in egg white and yolk are given in Fig. 3. The concentration of flubendazole residues on different days represents the individual pharmacokinetic of the animals.

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