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Simultaneous determination of triclabendazole and its metabolites in bovine and goat tissues by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A sensitive liquid chromatography–tandem mass spectrometry method for the simultaneous determination of triclabendazole, its main metabolites (triclabendazole sulphone and triclabendazole sulphoxide) and a marker residue (ketotriclabendazole) in bovine and goat muscle, liver, and kidney samples is developed and validated. Analyte extraction from samples is effectively performed using liquid–liquid extraction by acetonitrile. Chromatographic separation is performed on a C_{18} reversed-phase column with gradient elution. The analytes are detected by tandem quadrupole mass spectrometry after positive electrospray ionization by multiple reaction monitoring. The limits of detection for analytes are found to be 0.25–2.5 μg/kg in muscle tissues and 1–10 μg/kg in liver and kidney tissues, respectively. The recoveries of edible bovine and goat tissues range from 84.9% to 109.5% when spiked at different levels with analytes, with relative standard deviations generally below 12.8%.

1. Introduction

Triclabendazole (TCB) is a benzimidazole anthelmintic widely used to treat liver fluke infections in ruminants since its introduction in the early 1980s, due to its excellent activity against both adult and juvenile flukes [1,2].

TCB is normally given as an oral treatment. It is rapidly removed from portal blood by the liver and cannot be detected in the plasma as it is oxidized to the sulphoxide (TCB-SO) and sulphone (TCB-SO_2) metabolites, which are the main metabolites present in plasma [3,4]. Because of its toxic side effects [5] and the emergence of drug resistance [2,6,7], the clinical applications of TCB are strictly controlled in many countries. The EU [8], FAO/WHO [9], and Ministry of Agriculture of China have reported recommended maximum residue limits (MRLs) for TCB in edible ruminant tissues, and set the sum of extractable residues that may be oxidized to ketotriclabendazole (KETO) as a marker residue. In China, TCB was approved for use in ruminants, and the MRLs in muscle, liver, and kidney tissues of bovines were set at 200, 300, and 300 μg/kg, respectively. MRLs in the same tissues in goats were all set at 100 μg/kg [10]. Thus, it is of great importance to develop a sensitive method for the simultaneous determination and confirmation of the four residues in edible ruminant tissues.

Several methods have been published for the determination of TCB, TCB-SO, and TCB-SO_2 in milk [11,12] and in liver flukes [13]; TCB and TCB-SO_2 have been determined in animal liver as well [14]. Different techniques for analysis are used, such as HPLC–UV [11,13], HPLC–DAD [14], and UHPLC–MS/MS [12]. Recently, Jedziniak et al. [15] reported a screening method for the simultaneous determination of TCB, TCB-SO, TCB-SO_2, and KETO, as well as other benzimidazoles in milk by HPLC–MS. However, at present, no attempt has been made to simultaneously determine TCB, its metabolites (TCB-SO and TCB-SO_2), and a marker residue (KETO) in edible ruminant tissues, such as muscle, liver, and kidney using LC–MS/MS.

The aim of the present study is to develop a rapid, selective, and low-cost LC–MS/MS method to simultaneously determine TCB, TCB-SO, TCB-SO_2, and KETO with a simple preparation in edible bovine and goat tissues. Validation is conducted by determining method specificity, sensitivity, linearity, accuracy, precision, stability, and measurement uncertainty.

2. Experimental

2.1. Reagents and chemicals

TCB was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). TCB-SO_2, TCB-SO, KETO, and the internal standard (IS) TCB-D_3 were purchased from Witega Laboratories Berlin-Aldershof GmbH (Berlin, Germany). Chemical structures of the analytes are shown in Fig. 1. Acetonitrile and methanol were of HPLC grade

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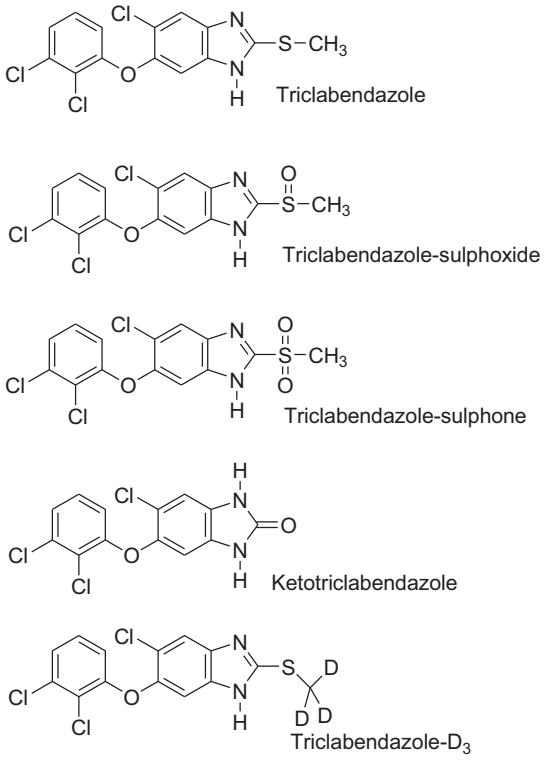

Fig. 1. Chemical structures of TCB, its main metabolites, the marker residue, and IS.

(Fisher, Fair Lawn, NJ, USA). Formic acid, n-hexane, and anhydrous sodium sulphate were of analytical grade and purchased from Aibi Chemical Industry (Shanghai, China). The water used in experiment was purified by a Milli-Q-water purification system (Millipore, Bedford, MA, USA) to HPLC grade.

2.2. Apparatus

The high-performance liquid chromatography–tandem mass spectrometry system (LC–MS/MS) consisted of a Waters Alliance 2695 HPLC system (Waters, Milford, MA, USA) and a Quattro Micro API triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray interface (ESI). Masslynx 4.1 software (Waters, Milford, MA, USA) was applied for system operation and data collection. A centrifuge (Anting Scientific Instrument, Shanghai, China) and N-Evap nitrogen evaporator (Organomation Associates, Berlin, MA, USA) were used during sample preparation.

2.3. Preparation of standard solutions

Stock standard solutions (500 μg/mL) of analytical standards were prepared by dissolving 5 mg of the compounds in 10 mL of methanol. The working standard solutions were prepared by serial dilution of the stock solution with a mobile phase (water containing 0.1% formic acid–acetonitrile, 55:45, *v/v*). The stock and working standard solutions were stable for 3 months at 4 °C.

2.4. Chromatographic conditions

An XTerra MS C_{18} HPLC column (2.1 mm × 100 mm, 3.5 μm) (Waters, Milford, MA, USA) equipped with a guard column (XTerra C_{18} 2.1 mm × 10 mm, 3.5 μm) (Waters, Milford, MA, USA) was used to separate TCB, its metabolites, and the marker residue in samples. The temperature of the column was set at 30 °C. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B). The gradient conditions are shown in Table 1. The flow rate during the complete runs was 0.2 mL/min.

Table 1
Chromatographic gradient conditions for separation of TCB, TCB-SO_2, TCB-SO, and KETO.

Time (min)	A (%)	B (%)	Flow rate (mL/min)
0.00	55	45	0.2
8.00	30	70	0.2
12.00	30	70	0.2
12.10	55	45	0.2
20.00	55	45	0.2

2.5. Mass spectrometry conditions

Mass spectrometer operating conditions were as follows: capillary voltage, 3.0 kV; source temperature, 100 °C; desolvation temperature, 300 °C. Nitrogen was used as a desolvation (400 L/h) and nebulizing gas (50 L/h). The parent molecular ions were fragmented in the collision cell with argon gas. Electrospray interface in the positive mode (ESI^+) was applied. The detection of analytes by tandem mass spectrometry was applied in the multiple reaction monitoring mode (MRM). A summary of the monitored ions and the optimized MS parameters for the examined analytes is shown in Table 2. Two transitions were followed for identification but only one was used for quantification.

2.6. Sample preparation

Edible ruminant tissue samples (bovine and goat muscle, liver and kidney samples) were purchased from local markets (Shanghai, China), homogenized in a high-speed food blender, and then stored at below −20 °C until analysis.

Samples (2 ± 0.01 g) were weighed into a 50 mL glass centrifuge tube. If necessary, TCB, TCB-SO_2, TCB-SO, KETO, and/or IS solutions were added. After mixing with a vortex mixer for 30 s and holding for 5 min at room temperature, 10 mL n-hexane was added to the centrifuge tube. The samples were centrifuged at 4000 rpm for 10 min. The upper n-hexane layer was discarded, and 5 mL of acetonitrile was added to the samples. After mixing in a vortex mixer for 1 min, samples were centrifuged at 4000 rpm for 10 min. This extraction procedure was repeated once with 5 mL of acetonitrile. The acetonitrile extracts were combined and transferred to a clean centrifuge tube. Five grams of anhydrous sodium sulphate was added to the combined supernatants, vortex-mixed for 1 min and then centrifuged at 4000 rpm for 10 min. The supernatant was transferred to a new tube and evaporated to dryness under nitrogen (60 °C). After cooling to room temperature, the dry residue was redissolved in 0.5 mL of the mobile phase (water containing

Table 2
The parameters and ion transformation of multiple reaction monitoring.

Compound	Parent ions (*m/z*)	Daughter ions (*m/z*)	Cone voltage (V)	Collision energy (eV)
KETO	328.9[a]	168.0[a]	55	29
	330.9	168.0	55	29
TCB	358.9[a]	343.9[a]	50	26
	360.9	345.9	50	26
TCB-SO	374.9[a]	356.9[a]	38	18
	376.9	358.9	38	18
TCB-SO_2	390.9[a]	242.0[a]	54	39
	392.9	242.0	54	39
TCB-D_3 (IS)	361.9	343.9	55	27

[a] Quantitative ion.

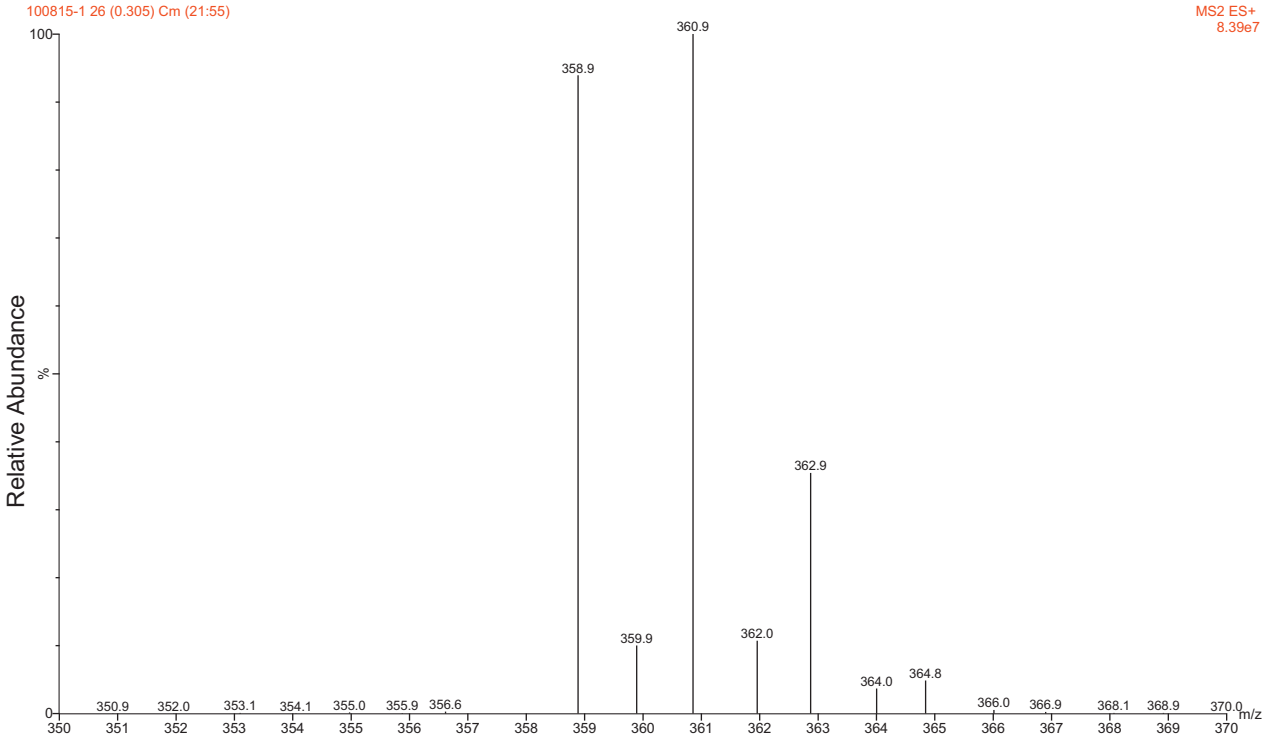

Fig. 2. The ions at *m/z* 358.9. 360.9, 362.9, and 364.8 in the positive MS mode for TCB represent the chlorine isotopes.

0.1% formic acid–acetonitrile 55:45, *v/v*), placed in an ultrasonic bath for 1 min, and then vortex-mixed for 30 s. After the cleaned sample extract was filtered through a 0.22 μm membrane filter, a 10 μL aliquot of the reconstituted solution was injected into the LC–MS/MS system for analysis.

2.7. Method validation

In this study, the linearity of the method was established through matrix-matched calibration curves spiked at six concentration levels for muscle samples (TCB-SO_2, TCB-SO, and KETO concentrations were set to 5, 10, 50, 100, 250, and 500 μg/kg, while TCB concentrations were set to 0.5, 1, 5, 10, 25, and 50 μg/kg), five concentration levels for liver and kidney samples (TCB-SO_2, TCB-SO, and KETO concentrations were set to 25, 50, 100, 250, and 500 μg/kg, while TCB concentrations were set to 2.5, 5, 10, 25, and 50 μg/kg).

The accuracy of the method was measured and expressed in terms of recovery. The recoveries were determined by comparing the calculated amounts of analytes in the samples (using matrix-matched calibration curves) with the total spiked amounts. The precision of the method was assessed by determining the intra- and inter-day relative standard deviation (RSD) of the analysis. Both recoveries and RSD (intra- and inter-day) were calculated from four analytes spiked at three different concentrations in six blank matrices. Accuracy and precision were evaluated by determining the recoveries of four analytes in spiked samples (bovine and goat muscle, liver, and kidney samples) using five replicates on three validation days.

The stability of a fortified sample was tested by freeze–thaw cycles. Three aliquot samples spiked at each of the low and high concentrations were stored at −20 °C for 24 h and then thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 12 h under the same conditions. The freeze–thaw cycle was repeated three times, after which the samples were analyzed by LC–MS/MS.

The limits of detection (LOD) and the quantification (LOQ) of TCB, TCB-SO, TCB-SO_2, and KETO were established by applying LC–MS/MS to blank samples ($n = 20$) fortified with the standards. LOD and LOQ were calculated as the concentrations corresponding to signals 3 and 10 times the standard deviation of the baseline noise, respectively.

3. Results and discussion

3.1. Chromatographic conditions

To achieve the efficient separation of TCB, TCB-SO_2, TCB-SO, and KETO, the effects of acetonitrile ratio in the initial mobile phase of gradient elution were investigated. To improve sensitivity, analyte separation and peak shape, trials were conducted by varying the concentration of the formic acid to 0%, 0.01%, 0.1%, and 0.5%. Higher signal intensities were obtained when 0.1% formic acid was used compared to other acid concentrations. At this concentration, optimum analyte separation and peak shape conditions were observed.

Under the chromatographic conditions described above, all analytes were eluted within 10 min. The retention times were 5.50, 6.10, 8.00, and 9.00 min for KETO, TCB-SO, TCB-SO_2, and TCB, respectively. The total elution run time was 20 min. From 12.10 to 20.00 min, the system was re-equilibrated by the initial composition of mobile phase.

3.2. Mass spectrometry conditions

The MS/MS parameters were previously tuned and optimized to ensure the maximum ion response of each analyte by direct infusion of a standard solution of 1 μg/mL with the syringe pump. ESI in both the positive and the negative ion modes were tested and the results showed that ESI in the positive ions mode (ESI^+) provided higher sensitivity. Since each analyte contains three chlorine atoms and chlorine has two stable isotopes (^{35}Cl and ^{37}Cl), all isotope peaks can be detected with ESI–MS. This pattern can be observed

in the MS spectrum of TCB (Fig. 2). The quartet with ions at *m/z* 358.9, 360.9, 362.9, and 364.8 in the MS mode for TCB represents the chlorine isotopes, i.e., the $^{35}Cl_3$, $^{35}Cl_2{}^{37}Cl$, $^{35}Cl^{37}Cl_2$, and $^{37}Cl_3$ versions of TCB as a deprotonated molecule, respectively. According to the natural occurrence of the different isotopes, TCB contains 45.3% $^{35}Cl_3$, 41.7% $^{35}Cl_2{}^{37}Cl$, 13.3% $^{35}Cl^{37}Cl_2$, and 1.4% $^{37}Cl_3$ [16]. For TCB, daughter ions in the MS/MS mode of *m/z* 358.9 and 360.9 were 343.9 and 345.9, respectively. Since the ions at *m/z* 358.9 and *m/z* 360.9 were the more abundant ions in the MS spectrum (Fig. 2), both MRM transitions for 358.9 > 343.9 and 360.9 > 345.9 can be used. In addition, to satisfy the identification criteria requested by 2002/657/EC Decision [17], two parent ions were selected for each compound in this study, and their daughter ions produced the strongest response to the qualitative and quantitative ions as ion pairs (Table 2). The detection of the analytes in the standard solution and spiked matrix samples was performed by the two different transitions. The determination of IS was based on one transition.

3.3. Sample preparation

Acetonitrile [11–13] and ethyl acetate [15] were found to be an effective solvent for extraction of TCB and its metabolites from biological samples. Acetonitrile was chosen as the extraction solvent because of its high extraction efficiency. Interfering residual fat particles were removed with n-hexane. In preliminary experiments, several solid phase extraction (SPE) approaches were tested for clean-up optimization. When the Waters OASIS® MCX and HLB SPE column clean-up procedures were, respectively, applied to purify in edible ruminant tissues, interfering matrix peaks were slightly reduced but still present in the chromatograms, and detection sensitivity was not improved. These results show that analytes extracted from edible ruminant tissues using MCX or HLB SPE columns alone cannot simultaneously provide sufficient recoveries for TCB, TCB-SO, TCB-SO_2, and KETO. This may be due to the large difference in polarity and pKa value between TCB and its metabolites. Moreover, avoiding clean-up by SPE can simplify the analyte extraction process. Therefore, in this method, liquid–liquid extraction using n-hexane and acetonitrile were employed to ensure sufficient sample clean-up and recovery.

3.4. Validation study

3.4.1. Identification and confirmation

To confirm the TCB listed in Group B of Annex I of Council Directive 96/23/EC, a minimum number of three identification points (IPs) was required [18]. The five IPs were obtained using LC–MS/MS with two parent ions (1 point each) and two daughter ions (1.5 points each) in two different transitions of the presented method. In terms of relative retention times, analyte peaks in the spiked samples were found to be within 2.5% tolerance compared to standards. Table 3 shows the ion ratios of two transitions of the four analytes in standard solutions and spiked samples together with the maximum permitted tolerances as listed in the 2002/657/EC Decision. The ion ratios of each analyte in the spiked samples fell within the maximum permitted tolerances for positive identifications. Thus, the performance criteria for confirmation were fulfilled.

Table 3
Ion ratios of two transition reactions of TCB, TCB-SO_2, TCB-SO, and KETO in standard solutions and spiked samples.

Analyte	Ion ratios of standard solutions	Maximum permitted tolerances[a]	Ion ratios of spiked samples
KETO	1.59	±20% (1.27–1.90)	1.38–1.79
TCB	1.01	±20% (0.81–1.21)	0.87–1.11
TCB-SO	1.00	±20% (0.80–1.20)	0.88–1.14
TCB-SO_2	1.46	±20% (1.17–1.75)	1.34–1.58

[a] According to 2002/657/EC Decision.

3.4.2. Specificity and sensitivity

To establish the specificity of the method, six individual samples fortified with analytes and IS as well as a non-fortified sample were analyzed. The results show that no significant interference was present in the chromatograms at the TCB, TCB-SO_2, TCB-SO, KETO, and IS retention times. Representative LC–MS/MS chromatograms of blank and spiked blank samples at 10 μg/kg for TCB, and 100 μg/kg for TCB-SO, TCB-SO_2, and KETO in bovine muscles are shown in Fig. 3. Since the chromatograms of edible bovine and goat tissues showed no significant differences, only the typical chromatograms of bovine muscle tissues are provided.

The values of LOQ and LOD in the present method are shown in Table 4. Due to the complexity of the liver and kidney samples, their LOQ and LOD values were significantly higher than those of the muscle sample.

3.4.3. Linearity

Response linearity was evaluated by matrix-matched calibration curves. To increase the fit for the highest concentrations, weighted least squares regression was applied with a weighting function of $1/x^2$. The regression coefficients (r^2) for the calibration curves obtained throughout all the tested concentrations for each analyte in this study were all above 0.990. The matrix spiked curves showed that the four analytes had a good linear fit within this range.

3.4.4. Accuracy and precision

The recoveries and RSD data for TCB, TCB-SO_2, TCB-SO, and KETO spiked in six matrices are summarized in Table 5. The mean recoveries values (n = 15) were between 84.9% and 109.5% for edible bovine tissues, and between 90.0% and 107.0% for edible goat tissues. The intra-day and inter-day variations, expressed as RSD, were less than 10.8% and 12.7% for edible bovine tissues, and 10.9% and 12.8% for edible goat tissues, respectively. The results demonstrate that the accuracy and precision of the present method were acceptable for routine monitoring purposes.

3.4.5. Stability

TCB, TCB-SO_2, TCB-SO, KETO, and TCB-D_3 (IS) were stable in stock solutions for at least 1 month at 4 °C. Stability results are shown in Table 6. TCB, its main metabolites, and KETO were shown

Table 4
Limits of quantification (LOQ) and detection (LOD) of the method in spiked samples.

Sample	TCB (μg/kg)		TCB-SO_2 (μg/kg)		TCB-SO (μg/kg)		KETO (μg/kg)	
	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD
Bovine muscle	0.5	0.25	5	2.5	5	2.5	5	2.5
Bovine liver	2.5	1	25	10	25	10	25	10
Bovine kidney	2.5	1	25	10	25	10	25	10
Goat muscle	0.5	0.25	5	2.5	5	2.5	5	2.5
Goat liver	2.5	1	25	10	25	10	25	10
Goat kidney	2.5	1	25	10	25	10	25	10

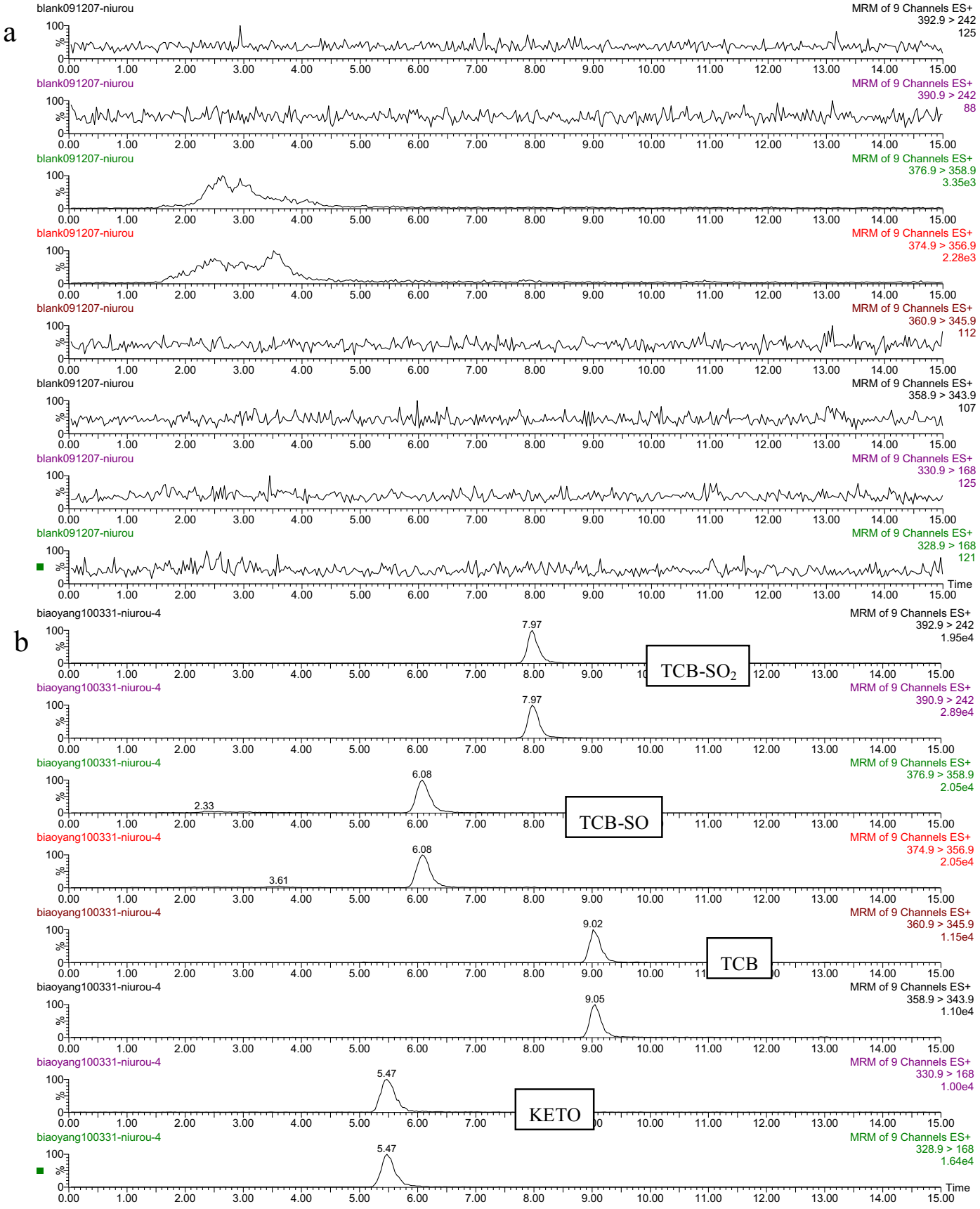

Fig. 3. Typical chromatograms for blank (a) and spiked muscles (b) of bovines with 10 μg/kg for TCB, and 100 μg/kg for TCB-SO, TCB-SO_2, and KETO, acquired by multiple reaction monitoring.

Table 5
Recovery and precision results for determination of TCB, TCB-SO_2, TCB-SO, and KETO in edible ruminant tissues (n = 5 at each concentration level, on 3 separation days).

Sample	Compound	Spiked level (μg/kg)	Mean recovery (%)	Intra-day RSD (%)	Inter-day RSD (%)	U% (k = 2)
Bovine muscle	TCB	5–10–25	102.3–101.6–100.1	4.5–2.8–1.6	5.1–2.3–2.7	12.7
	TCB-SO_2	50–100–250	109.5–106.7–101.0	3.5–1.3–4.0	5.2–3.1–7.9	20.8
	TCB-SO	50–100–250	107.8–107.5–100.6	2.8–2.6–4.0	5.8–3.3–8.5	22.3
	KETO	50–100–250	106.6–105.8–103.7	4.0–1.9–5.1	4.8–5.0–6.5	20.0
Bovine liver	TCB	5–10–25	90.8–94.4–99.1	7.1–6.9–6.7	6.5–5.8–8.0	22.5
	TCB-SO_2	50–100–250	96.4–94.4–90.5	9.4–3.7–4.2	11.4–8.0–11.2	33.5
	TCB-SO	50–100–250	84.9–91.1–93.4	6.0–10.8–10.0	8.7–9.5–12.0	31.2
	KETO	50–100–250	93.4–93.0–87.3	10.7–8.5–3.7	8.7–9.4–11.2	30.8
Bovine kidney	TCB	5–10–25	101.8–96.4–100.2	2.0–3.3–2.7	3.9–5.9–3.9	15.9
	TCB-SO_2	50–100–250	100.1–102.5–106.7	7.3–6.7–5.1	8.9–12.5–9.7	37.4
	TCB-SO	50–100–250	96.6–99.3–104.5	9.0–9.0–8.5	12.1–9.1–7.5	33.6
	KETO	50–100–250	102.4–100.8–102.4	4.4–6.4–4.1	8.1–12.7–8.7	35.4
Goat muscle	TCB	5–10–25	100.1–99.3–99.1	3.8–3.3–1.7	6.8–6.5–6.3	22.5
	TCB-SO_2	50–100–250	103.8–104.0–103.8	7.3–6.4–2.2	7.9–6.4–10.9	31.0
	TCB-SO	50–100–250	101.3–103.2–100.6	6.6–4.1–2.7	9.1–5.7–7.5	26.6
	KETO	50–100–250	106.1–104.8–107.0	7.3–3.8–1.8	7.0–4.2–7.8	23.9
Goat liver	TCB	5–10–25	103.4–100.6–102.8	4.5–5.2–0.9	6.6–7.1–5.6	22.8
	TCB-SO_2	50–100–250	100.9–97.1–96.9	5.5–5.3–3.1	8.7–8.5–9.5	30.2
	TCB-SO	50–100–250	103.9–106.0–101.9	5.4–5.1–5.8	6.9–3.5–6.5	20.9
	KETO	50–100–250	102.6–98.3–95.8	4.5–3.7–4.6	7.0–9.9–12.8	34.5
Goat kidney	TCB	5–10–25	93.2–98.4–96.8	3.5–8.1–3.7	9.0–7.4–4.5	24.0
	TCB-SO_2	50–100–250	101.8–102.4–100.0	9.4–3.5–2.6	8.5–5.6–7.7	25.9
	TCB-SO	50–100–250	90.0–98.2–99.2	8.5–9.0–8.5	9.1–6.2–6.0	23.6
	KETO	50–100–250	105.8–103.2–105.3	7.9–10.9–6.8	5.7–9.1–4.9	24.5

Table 6
Stability of compounds in spiked samples after three freeze–thaw cycles (n = 3 for each concentration level).

Compound	Spiked level (μg/kg)	Recovery (%) (RSD%)					
		Bovine muscle	Bovine liver	Bovine kidney	Goat muscle	Goat Liver	Goat kidney
TCB	5	100.7 (6.3)	110.3 (2.0)	114.3 (1.3)	106.5 (5.5)	101.7 (5.5)	117.8 (9.3)
	50	109.3 (5.6)	111.0 (0.5)	115.1 (3.5)	103.3 (3.4)	108.4 (6.0)	99.7 (9.4)
TCB-SO_2	50	106.2 (3.6)	102.9 (2.3)	92.2 (9.3)	93.3 (4.0)	111.0 (4.3)	105.7 (0.9)
	500	104.6 (5.9)	105.7 (3.3)	111.4 (9.6)	96.0 (6.8)	110.1 (6.7)	96.8 (6.4)
TCB-SO	50	83.6 (5.0)	92.5 (3.2)	89.2 (9.9)	85.5 (6.6)	102.0 (5.1)	80.4 (0.5)
	500	87.8 (4.8)	102.7 (8.7)	98.8 (5.7)	94.4 (2.2)	103.4 (12.0)	81.3 (3.4)
KETO	50	99.7 (12.3)	106.9 (7.1)	96.7 (16.1)	92.9 (9.6)	101.6 (11.9)	112.4 (4.7)
	500	106.4 (4.9)	108.1 (6.2)	105.1 (7.0)	96.5 (1.5)	103.2 (9.5)	107.4 (2.2)

to remain stable in the muscle, liver, and kidney samples of bovines and goats after three freeze–thaw cycles.

3.4.6. Measurement uncertainty

For further validation, the measurement uncertainty (MU) for all analytes was calculated. This approach of using the within-laboratory reproducibility as a good estimate of the combined MU is taken from the SANCO/2004/2726 rev1 document [19]. According to the demonstrations of Wang and Wotherspoon [20] and Maroto et al. [21], the MU can be determined by calculating the within-laboratory reproducibility of the method. For the calculation of the extended uncertainty, a coverage factor is required. The expanded uncertainty was calculated using a coverage factor (k) of 2. The calculated expanded uncertainties as relative values (U%) for each analyte are given in Table 5.

4. Conclusion

This paper reports the development and validation of an LC–MS/MS method for the simultaneous determination of TCB, TCB-SO_2, TCB-SO, and KETO in edible bovine and goat tissues. The four analytes were extracted from samples using a relatively rapid and simple LLE procedure by acetonitrile. This method met the regulatory requirement of the confirmatory criteria according to 2002/657/EC Decision by five IPs obtained for each analyte. The proposed method is useful for the control of TCB residues in edible bovine and goat tissues.

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