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ARTICLE

Multiclass Method for the Determination of Anthelmintic and Antiprotozoal Drugs in Livestock Products by Ultra-High-Performance Liquid Chromatography–Tandem Mass Spectrometry

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Abstract   The objective of this study was to establish a multi-residue quantitative method for the analysis of anthelmintic and antiprotozoal drugs in various livestock products (beef, pork, and chicken) using ultra-high-performance liquid chromatography–tandem mass spectrometry. Each compound performed validation at three different levels i.e., 0.5, 1, and 2× the maximum residue limit according to the CODEX guidelines (CAC/GL 71-2009). This study was conducted according to the modified quick, easy, cheap, effective, rugged, and safe procedure. The matrix-matched calibrations gave correlation coefficients >0.98, and the obtained recoveries were in the range of 60.2%–119.9%, with coefficients of variation ≤32.0%. Furthermore, the detection and quantification limits of the method were in the ranges of 0.03–3.2 and 0.1–9.7 μg/kg, respectively. Moreover, a survey of residual anthelmintic and antiprotozoal drugs was also carried out in 30 samples of beef, pork, and chicken collected in Korea. Toltrazuril sulfone was detected in all three samples. Thus, our results indicated that the developed method is suitable for determining the anthelmintic and antiprotozoal drug contents in livestock products.

Keywords   anthelmintic, antiprotozoal, livestock products, ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS), multi-class analysis

Introduction

With the consumption of livestock products increasing annually, veterinary drugs are being increasingly employed to promote growth and prevent and treat disease (Zeleny et al., 2006). If these drugs or their metabolites are not fully excreted, consuming the derived animal products can lead to potential health risks for the consumers. To address this issue, the residual tolerance standards of such compounds are strictly regulated (Beyene, 2016). Generally, such residues are generated because of excessive use or noncompliance with the withdrawal period (Danaher et al., 2007; Delatour et al., 1981;

Whittaker and Faustman, 1992). Veterinary medicines include antibiotics, synthetic antibacterial agents, nervous system drugs, hormones, anticoccidial drugs, antimicrobial agents, and anthelmintics (Rana et al., 2019). Despite their advantages, antibiotics have been reported to lead to the generation and propagation of resistant bacteria, in addition to the induction of hypersensitivity reactions, tumor induction, abnormal physical development, and teratogenesis (Abbas et al., 2011; González-Díaz et al., 2005). Thus, maximum residue limits (MRLs) have been set for 193 substances in Korea, including 26 banned substances. For example, the number of MRLs of the anthelmintic and antiprotozoal drugs are 26 and 23, respectively (MFDS, 2023).

As one example drug class, anthelmintic drugs are used to treat parasites (Danaher et al., 2007). More specifically, the benzimidazoles (e.g., albendazole, cambendazole, carbendazim, febantel, flubendazole, oxfendazole, oxibendazole, mebendazole, thiabendazole, and triclabendazole) are widely used in agriculture (Cano et al., 1987). In addition, avermectin is a macrocyclic lactone anthelmintic agent produced by *Streptomyces avermitiles*. To broaden its therapeutic range, the original structure of avermectin has been modified by substitution to give abamectin, ivermectin, doramectin, and eprinomectin. In this group of compounds, abamectin is used as an insecticide, and its side effects include psychosis, respiratory failure, and hypotension (Wang et al., 2009). In addition, ivermectin is a hydrogenated version of abamectin that is effective in treating onchocerciasis, despite causing various side effects, such as a rash, swelling, headache, and dizziness (Hoyos et al., 2017).

In contrast, antiprotozoal drugs are used to treat protozoan infections. In particular, coccidiostats are used to prevent or treat coccidiosis, which is a disease caused by protozoan parasites that parasitize and attack the digestive tract of animals, causing diarrhea and secondary infections such as enteritis (Roila et al., 2019; Rusko et al., 2019).

According to previous studies, anthelmintic and antiprotozoal drugs frequently exceed the MRL. For example, Lee et al. (2017) confirmed MRL violations in pigs treated with mebendazole, while Escribano et al. (2012) confirmed an excess of ivermectin in the liver and milk of cattle, sheep, pigs, and rabbits. Moreover, Cooper et al. (2012) reported that the MRLs of rafoxanide and doramectin were violated in pigs. Thus, the development of novel methods with high sensitivities and resolutions is required due to the frequent occurrences of anthelmintic and antiprotozoal drugs in animal samples. In this paper shows excellent sensitivity compared to other studies (Clarke et al., 2013; Kang et al., 2014; Kang et al., 2015). To ensure domestic food safety in Korea, The Ministry of Food and Drug Safety is preparing to introduce a positive list system (PLS). The PLS Program for veterinary drugs covers livestock and fishery products produced in 2024 or beyond. Previously, the CODEX guidelines (CAC/GL 71-2009) were applied in cases where no MRL had been previously established in Korea; alternatively, the lowest MRL established for similar products was employed. However, with the introduction of the PLS, a limit of 10 μg/kg is applied if a Korean MRL is unavailable. Therefore, a rapid, highly sensitive, and reliable analytic method is required to prepare for the introduction of the PLS. Among the various analytical techniques reported to date, ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) has become a popular technique for analyzing veterinary drugs owing to its ability to analyze a wide range of compounds at low levels quickly (Moloney et al., 2012). In addition, according to recent study trends, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method has been used to develop multi-residue analytical approaches for analyzing pesticides and veterinary drugs in various matrices. The QuEChERS approach is flexible and can be modified depending on the matrix and the properties of the analyte. This method is beneficial because it minimizes the time required to complete the extraction and cleanup processes, while also reducing the cost of analysis (Chen et al., 2021; Kang et al., 2014; Stubbings and Bigwood, 2009; Ye et al., 2022).

Thus, by applying a modified QuEChERS approach, this study aims to increase the extraction efficiency by adding anhydrous magnesium sulfate (MgSO$_4$) and sodium chloride (NaCl) to remove moisture and interfering substances from the

sample. This is followed by the separation of the extraction solution and the aqueous layer using the salting-out method. Furthermore, during the dispersive solid-phase extraction (d-SPE) step, MgSO₄, primary secondary amine (PSA), and C₁₈ are used for matrix cleanup. Consequently, this study aims to verify the sensitivity and quantitation of 54 anthelmintic and antiprotozoal drugs that are commonly present in livestock products, and this will be achieved using a modified QuEChERS extraction and purification approach, followed by UHPLC-MS/MS analysis.

Materials and Methods

Chemicals and reagents

The following standards were purchased from Sigma-Aldrich (St. Louis, MO, USA): 5-hydroxy thiabendazole, albendazole sulfoxide, bithionol, carbendazim, fluazuron, keto triclabendazole, isometamidium, ternidazole, thiophanate, and toltrazuril sulfone. Arprinocide, benznidazole, diethylcarbamazine, and halofuginone were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Emamectin b_{1a} (emamectin) and ornidazole were purchased from ChemService (West Chester, PA, USA) and StordSynthesis (Hebei, China), respectively. The rest of the 42 compounds (abamectin, albendazole, albendazole sulfone, etc.) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Methanol (MeOH) and acetonitrile (MeCN) were purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO), formic acid, MgSO₄, and NaCl were purchased from Sigma-Aldrich, and PSA was purchased from Agilent Technologies (Santa Clara, CA, USA). Ammonium formate was purchased from Alfa Aesar (Ward Hill, MA, USA) and C₁₈ (55–105 μm, 125 Å) was purchased from Waters (Milford, MA, USA). A syringe filter from Teknokroma (Barcelona, Spain) was used by incorporating it into polytetrafluoroethylene (PTFE) membrane filters (0.2 μm). For albendazole, albendazole sulfone, albendazole sulfoxide, buquinolate, flubendazole, oxfendazole, oxfendazole sulfone, oxibendazole, mebendazole, mebendazole amine, standard solutions (1,000 μg/mL) were prepared in MeOH/DMSO (1:1, v/v). Similarly, MeCN was used as the solvent to prepare a standard stock solution of guaifenesin (1,000 μg/mL), while DMSO was used to prepare the stock solutions for fenbendazole, 5-hydroxy mebendazole, methylbenzoquate, and nicarbazin (1,000 μg/mL). The corresponding standard stock solutions (1,000 μg/mL) were prepared at MeOH in all other compounds. All standard stock solutions were stored in amber bottles at −20℃.

Sample collection and preparation

Beef (n=10), pork (n=10), and chicken (n=10) were purchased from local markets in Korea. Each sample was homogenized and stored in a freezer (−20℃) until required for further use. Thus, each homogenized sample (2 g) was weighed into a 50 mL centrifuge tube and then extracted using 0.1% formic acid in MeCN/MeOH (95:5, v/v, 10 mL) and water (10 mL) under shaking for 5 min. Subsequently, MgSO₄ (4 g) and NaCl (1 g, original QuEChERS salt) were added to the sample. After, shaken for 5 min, and subjected to centrifugation at 4,700×g (4℃, 10 min). The supernatant was then transferred to a 50 mL centrifuge tube containing C₁₈ (150 mg), PSA (150 mg), and MgSO₄ (900 mg). And then, the obtained mixture was shaken for 5 min and centrifuged at 4,700×g (4℃, 5 min). The obtained supernatant (5 mL) was transferred to a new centrifuge tube, DMSO (20 μL) was added, and the solvent was evaporated under a stream of N₂ at 40℃. Afterwards, the residue was dissolved in a mixture of MeOH and water (1:1, v/v, 1 mL), and the extract was subsequently filtered through a 0.2 μm PTFE filter before analysis (Kim et al., 2021).

Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) conditions

Separation was conducted on a Shimadzu UHPLCMS 8060 triple quadrupole mass spectrometer (MS, Shimadzu, Kyoto,

Japan) equipped with a Waters X-SELECT HSS C$_{18}$ column (2.1 mm×150 mm, 3.5 μm particle size, Waters, Dublin, Ireland). Data processing used LC solution software version (5.99) from Shimadzu. Gradient separation was performed using a binary gradient composed of water containing 0.1% formic acid and 2 mM ammonium formate (mobile phase A) and MeCN containing 0.1% formic acid (mobile phase B). The gradient profile was as follows: 0 min, 15% B; 2 min, 15% B; 12.5 min, 95% B; 17.0 min, 95% B; 17.1 min, 15% B; 20.0 min, 15% B. The injection volume was 5 μL, and a flow rate of 0.3 mL/min was used under argon gas. The MS source settings were as follows: capillary voltages=4.0 kV (positive) and 2.8 kV (negative); capillary temperature=350℃, auto-sampler temperature=15℃, column temperature=40℃, and cone voltage=30 kV. The MS instrument was operated in the electrospray ionization (ESI) mode with positive and negative switching modes, and scheduled multiple reaction monitoring (MRM) was employed for all target compounds.

Method validation

The linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) of the developed method were determined according to the CODEX guidelines (CAC/GL71-2009) and the Ministry of Food and Drug Safety (MFDS) of Korea guidelines (FAO and WHO, 2009; MFDS, 2016). More specifically, the accuracy and precision were determined by analyzing negative samples at three different concentrations, i.e., spiking at 0.5, 1, and 2× the MRL. In addition, the analysis included the determination of toltrazuril (toltrazuril sulfone), emamectin (emamectin B1a), and nicarbazin [N,N'-bis(4-nitrophenylurea)], based on the specified marker residues. The matrix-matched standards for the calibration curves were prepared using a six-point range of target concentrations (i.e., 0.25, 0.5, 1, 2, 4, and 8× the MRL). The LODs and LOQs were defined as the concentrations at which the signal-to-noise ratios were ≥3 and ≥10, respectively.

Matrix effect

To determine the degree of the matrix effect for each system, the matrix-matched curve of a post-extraction spiked sample and the solvent standard curve were compared at the same concentration (in the case set MRL value; 0.25, 0.5, 1, 2, 4, and 8× the MRL and in the case not set MRL value; 2.5, 5, 10, 20, and 40 μg/kg), as outlined in Eq. (1) below. In general, the matrix components of a sample can either increase or decrease ionization efficiency due to interfering substances, i.e. salts, lipids, and peptides (Antignac et al., 2005).

$$ME(\%) = \left(\frac{Slope_{matrix\ matched\ standard\ curve}}{Slope_{solvent\ standard\ curve}} - 1 \right) \times 100 \tag{1}$$

Results and Discussion

Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) optimization

The MS parameters were determined using individual standard solutions and were optimized based on the mass spectra of all compounds. Using the ESI mode with positive and negative switching and MRM, bithionol, chlorfluazuron, oxyclozanide, keto triclabendazole, nicarbazine, niclosamide, and toltrazuril sulfone were detected as their corresponding [M−H]$^-$ species, semduramyicin was detected as [M+NH]$^+$, and all other compounds were detected as [M+H]$^+$ mode. Using standard solutions diluted in MeOH/water (1:1, v/v), the MS parameters were optimized using a cone voltage of 30 V. The parent and daughter ions were selected by optimizing the collision energy. Furthermore, daughter ions with higher intensities and better peak

shapes were selected as quantitative ions. Most compounds possessed one parent ion and either one or two daughter ions. The optimized precursor ions, daughter ion collision energies, and retention times of all compounds are listed in Table 1. A reversed-phase X-SELECT HSS C_{18} column was used to separate the various veterinary drugs examined herein. This column was selected because the multi-residue analysis was previously performed using a C_{18} column (Dasenaki and Thomaidis, 2015). It was found that mobile phase A improved sensitivity and reduced peak tailing (Chang et al., 2019; Clarke et al., 2013; Frenich et al., 2014), while mobile phase B produced a better peak shape than a mixture of MeOH and 0.1% formic acid in MeCN (Zrnčić et al., 2014). Supplementary Fig. S1 shows the extracted ion chromatograms of the target compounds. These chromatograms were observed by injecting an aliquot (5 μL) of the desired standard solution into the beef sample at a concentration of 100 μg/kg (fenbantel and isometamidium) or 10 μg/kg, which corresponds to spiking of 1× the MRL.

Table 1. UHPLC-MS/MS parameters of the 54 target compounds

Class	Compounds	ESI (+/−)	Molecular weight (m/z)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)	Retention time (min)
Anthelmintic	Abamectin	+	872.5	895.0	327.3[a]	20	13.8
					449.3	20	
					751.4	20	
	Albendazole	+	265.1	266.0	234.0[a]	20	8.00
					191.1	20	
					159.2	20	
	Albendazole sulfoxide	+	281.1	282.0	208.1[a]	16	5.12
					240.1	19	
					159.2	19	
	Albendazole sulfone	+	297.1	298.0	159.2[a]	20	6.19
					224.1	20	
					266.1	20	
	2-Amino albendazole sulfone	+	239.1	240.0	133.2[a]	17	2.84
					198.2	16	
					105.2	30	
	Benznidazole	+	260.1	261.0	91.2[a]	20	6.51
					107.3	18	
					65.3	17	
	Bithionol	−	353.9	352.0	161.1[a]	−25	12.43
					192.0	−25	
					125.1	−42	
	Cambendazole	+	302.1	303.0	217.0[a]	20	6.15
					261.0	20	
					190.1	24	
	Carbendazim	+	191.1	192.0	132.2[a]	20	2.78
					105.2	30	
					160.1	30	

Table 1. UHPLC-MS/MS parameters of the 54 target compounds (continued)

Class	Compounds	ESI (+/−)	Molecular weight (m/z)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)	Retention time (min)
	Carnidazole	+	244.1	245.0	118.2[a]	30	6.51
					75.2	14	
					47.2	26	
	Chlorfluazuron	−	539.0	538.0	518.0[a]	−15	13.1
					355.0	−22	
					175.1	−42	
	Cymiazole	+	218.1	219	171.2[a]	15	6.00
					144.1	20	
					77.3	23	
	Derquantel	+	479.6	481	405.2[a]	20	7.10
					462.1	20	
					148.3	30	
	Diethylcarbamazine	+	199.2	200.0	100.0[a]	20	1.79
					72.0	20	
					44.2	20	
	Emamectin	+	885.5	886.0	158.1[a]	30	10.98
					82.2	34	
					159.2	32	
	Febantel	+	446.1	448.0	384.1[a]	19	10.50
					416.2	14	
					281.2	33	
	Fenbendazole	+	299.1	299.9	268.0[a]	20	8.90
					159.0	35	
					131.3	46	
	Fluazuron	+	505.0	506.0	158.2[a]	22	12.50
					141.2	49	
					351.1	21	
	Flubendazole	+	313.1	314.0	282.1[a]	21	7.90
					123.2	35	
					95.2	50	
	2-Amino flubendazole	+	255.1	256.0	123.2[a]	34	6.00
					95.2	34	
					123.2	17	
	Levamisole	+	204.1	205.0	91.0[a]	20	2.91
					123.1	20	
					178.0	20	

Table 1. UHPLC-MS/MS parameters of the 54 target compounds (continued)

Class	Compounds	ESI (+/−)	Molecular weight (m/z)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)	Retention time (min)
	Mebendazole	+	295.1	295.9	264.0[a]	20	7.55
					105.2	30	
					77.2	20	
	Mebendazole amine	+	237.1	238.0	105.2[a]	16	5.71
					133.3	30	
					77.2	16	
	5-Hydroxy mebendazole	+	297.1	298.0	266.0[a]	20	5.88
					79.2	20	
					160.2	20	
	Morantel	+	220.1	221.0	123.0[a]	20	5.58
					164.0	20	
					111.0	21	
	Niclosamide	−	326.0	325.0	171.1[a]	−20	11.22
					289.0	−18	
					135.1	−21	
	Ornidazole	+	219.0	220.0	128.2[a]	10	5.29
					82.3	20	
					42.2	10	
	Oxantel	+	216.1	217.0	91.1[a]	20	3.08
					118.3	20	
					131.3	29	
	Oxfendazole	+	315.1	316.0	159.2[a]	19	6.23
					284.2	21	
					191.2	21	
	Oxfendazole sulfone	+	331.1	332.0	300.1[a]	19	7.23
					159.2	12	
					131.3	12	
	Oxibendazole	+	249.1	250.0	176.0[a]	20	6.57
					218.0	20	
					148.2	18	
	Oxyclozanide	−	398.9	397.0	362.0[a]	−19	11.08
					202.0	−24	
					176.2	−27	
	Praziquantel	+	312.2	313.0	159.0[a]	20	8.97
					131.3	21	
					174.2	19	

Table 1. UHPLC-MS/MS parameters of the 54 target compounds (continued)

Class	Compounds	ESI (+/−)	Molecular weight (m/z)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)	Retention time (min)
	Pyrantel	+	206.1	207.0	109.0[a]	20	4.25
					150.0	34	
					136.0	34	
	Ternidazole	+	185.1	186.0	82.2[a]	30	2.87
					128.2	12	
					42.2	12	
	Tetramisole	+	204.1	205.0	178.0[a]	20	2.91
					91.0	20	
					123.1	20	
	Thiabendazole	+	201.0	202.0	121.2[a]	19	3.20
					175.0	20	
	5-Hydroxy thiabendazole	+	217.0	218.0	131.0[a]	20	1.92
					65.2	26	
					191.1	16	
	Thiophanate	+	370.1	371.0	147.2[a]	16	9.11
					81.3	15	
					151.0	20	
	Triclabendazole	+	358.0	359.0	273.9[a]	35	10.99
					343.9	30	
					171.1	53	
	Keto triclabendazole	−	328.0	326.9	182.1[a]	−25	9.63
					146.1	−34	
					118.0	−40	
Antiprotozoal	Arprinocid	+	277.1	278.0	142.9[a]	20	6.01
					107.2	16	
					108.2	19	
	Buquinolate	+	361.2	362.0	204.0[a]	20	10.05
					316.1	21	
					148.2	25	
	Diaveridine	+	260.1	261.0	123.1[a]	20	2.84
					81.3	20	
					245.0	19	
	Guaifenesin	+	198.1	199.0	121.2[a]	15	5.30
					125.3	25	

Table 1. UHPLC-MS/MS parameters of the 54 target compounds (continued)

Class	Compounds	ESI (+/−)	Molecular weight (m/z)	Precursor ion (m/z)	Production (m/z)	Collision energy (eV)	Retention time (min)
	Halofuginone	+	413.0	415.8	100.2[a]	29	6.19
					120.2	20	
					138.3	20	
	Imidocarb	+	348.2	349.0	188.2[a]	20	1.54
					162.2	13	
					97.7	24	
	Isometamidium	+	460.2	461.0	299.2[a]	10	5.70
					313.2	16	
					314.3	30	
	Methylbenzoquate, Nequinate	+	365.2	366.0	334.1[a]	20	9.88
					91.0	22	
					201.1	26	
	Monensin	+	692.4	693.0	675.4[a]	24	16.60
					461.4	24	
					479.3	24	
	Nicarbazine	−	302.1	301.0	136.9[a]	−20	9.74
					107.1	−30	
					46.0	−20	
	Semduramicin	+	872.5	890.0	629.4[a]	34	14.49
					647.4	20	
					727.5	34	
	Tinidazole	+	247.1	248.0	121.2[a]	19	4.30
					82.2	11	
					128.2	11	
	Toltrazuril sulfone	−	457.1	456.0	41.8[a]	−22	9.96
					399.1	−22	

[a] The quantification ions.
UHPLC-MS/MS, ultra-high-performance liquid chromatography–tandem mass spectrometry; ESI, electrospray ionization.

Sample preparation

This study was conducted according to the modified QuEChERS procedure. The sample extraction and clean-up conditions were optimized based on a previously used method for multiclass drug analysis (Kim et al., 2021). Kim et al. used a modified QuEChERS type extraction method and added the concentration step. The original QuEChERS method consists of two steps an extraction/partitioning step with the addition of salts, and a clean-up step that uses d-SPE. More specifically, sample extraction was carried out using 0.1% formic acid with MeCN/MeOH (95:5, v/v), which had been previously demonstrated to yield a high recovery rate (Clarke et al., 2013; Lopes et al., 2012). In addition, $MgSO_4$ was employed due to its good water

absorbency properties, which permits salting-out, while NaCl was added to increase the polarity of the extraction solvent and enhance the extraction selectivity (Rejczak and Tuzimski, 2015). For sample clean-up, PSA was used to remove fatty acids and organic acids, $MgSO_4$ was used to remove water, and a C_{18} absorbent was used to remove non-polar components (Anastassiades et al., 2003; Wilkowska and Biziuk, 2011). DMSO was added prior to sample concentration to enhance the sample recovery to act as a keeper during evaporation (Kim et al., 2021; Whelan et al., 2010).

Validation of the analytical method

Method validation was performed in terms of linearity, accuracy, precision, LOD, and LOQ. All compounds exhibited a best linearity, with correlation coefficients (r^2) exceeding 0.98 at matrix-matched calibration at six points. The accuracy, expressed as the recovery, ranged from 60.2% to 119.9%, and the coefficients of variation (CV) ranged from 1.2% to 31.5% for the three determined levels. In the chicken samples, the average recovery of 2-amino albendazole sulfone was ~111.1%, which was considered unacceptable based on the recovery limit of 110% specified by the CODEX guidelines at a concentration of 200 μg/kg; all other compounds satisfied the CODEX guidelines. In addition, inter-laboratory (n=2) validation was conducted according to CODEX guidelines (CAC/GL-71) and the results were satisfied with the guideline. Table 2 lists the accuracies and precisions obtained of all compounds following their analyses in the three matrices. In addition, the LOD values ranged from 0.3 to 3 μg/kg, while the LOQ values ranged from 1 to 10 μg/kg, which are lower than the corresponding values of the Korean MRLs. It should be noted that, in general, the LOQ values were ~1 μg/kg; however, the corresponding values for imidocarb and pyrantel in beef were 10 μg/kg, respectively, while oxfendazole had a LOQ value of 10 μg/kg. The LOD, LOQ, and Korean MRL values for the three matrices (beef, pork, and chicken) are given in Table 3, wherein it can be deduced that the obtained values were satisfactory. Thus, the developed method appeared to demonstrate an acceptable analytical performance for residue control in livestock products.

Table 2. Validation of the analytical method for the 54 target compounds (n=5)

Compound	Beef				Pork				Chicken			
	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r^2	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r^2	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r^2
Abamectin	5	97.3	27.9	0.9914	5	75.6	25.6	0.9865	5	94.1	22.8	0.9838
	10	102.0	19.1		10	73.2	24.3		10	105.7	16.4	
	20	91.8	19.5		20	98.1	17.7		20	105.1	11.6	
Albendazole	50	77.6	12.2	0.9990	50	90.3	7.9	0.9970	50	107.8	9.1	0.9952
	100	73.8	12.3		100	97.0	9.0		100	111.2	11.7	
	200	93.2	6.4		200	94.6	11.5		200	109.0	12.6	
Albendazole sulfone	50	109.4	13.4	0.9946	50	107.0	16.3	0.9944	50	109.0	5.9	0.9987
	100	110.8	20.1		100	91.0	18.6		100	107.3	8.6	
	200	98.8	10.4		200	94.0	11.9		200	93.6	13.6	
Albendazole sulfoxide	50	96.3	9.2	0.9985	50	85.5	11.2	0.9996	50	110.1	10.6	0.9996
	100	92.2	10.0		100	93.8	8.3		100	110.0	9.1	
	200	85.9	3.9		200	93.3	1.2		200	107.2	4.3	

Table 2. Validation of the analytical method for the 54 target compounds (n=5) (continued)

Compound	Beef				Pork				Chicken			
	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r^2	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r^2	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r^2
2-Amino albendazole sulfone	50	106	4.8	0.9979	50	100.1	3.6	0.9999	50	113.8	7.2	0.9998
	100	100.8	7.5		100	100.5	9.7		100	111.7	5.8	
	200	85.7	18.0		200	99.7	2.8		200	111.1	7.0	
Arprinocid	5	106.6	4.7	0.9974	5	92.0	5.2	0.9996	5	105.3	5.4	0.9984
	10	87.4	8.2		10	98.3	5.1		10	111.6	3.0	
	20	88.9	6.6		20	90.5	6.6		20	109.3	5.7	
Benznidazole	5	92.7	5.9	0.9969	5	98.5	5.2	0.9991	5	116.0	8.9	0.9996
	10	92.4	7.8		10	96.5	5.6		10	115.9	6.2	
	20	92.8	6.4		20	93.2	6.0		20	112.0	6.5	
Bithionol	5	71.0	6.4	0.9983	5	79.4	21.0	0.9966	5	104.2	16.1	0.9854
	10	76.9	14.9		10	90.3	11.4		10	116.9	18.0	
	20	98.5	13.0		20	112.4	17.5		20	115.0	18.2	
Buquinolate	5	93.5	16.2	0.9922	5	90.6	12.5	0.9960	5	90.8	7.9	0.9987
	10	97.6	10.4		10	83.5	23.0		10	107.9	4.2	
	20	100.6	11.1		20	82.6	11.7		20	81.9	10.1	
Cambendazole	5	99.8	10.3	0.9944	5	60.9	28.5	0.9944	5	105.9	16.2	0.9914
	10	74.1	16.7		10	100.4	8.1		10	115.4	9.8	
	20	86.4	7.2		20	102.9	9.9		20	114.3	11.5	
Carbendazim	5	107.2	10.5	0.9995	5	75.3	14.7	0.9976	5	110.8	8.3	0.9995
	10	119.9	11.0		10	100.4	7.4		10	102.4	11.8	
	20	104.8	8.0		20	102.5	10.9		20	101.9	6.0	
Carnidazole	5	99.8	9.2	0.9990	5	96.4	8.2	0.9995	5	101.6	8.9	0.9998
	10	93.4	11.3		10	103.0	4.8		10	99.3	2.8	
	20	98.4	11.7		20	92.0	7.2		20	99.5	9.0	
Chlorfluazuron	5	103.5	11.1	0.9946	5	118.6	11.9	0.9944	5	101.2	8.6	0.9962
	10	89.7	25.0		10	81.6	6.3		10	103.5	19.0	
	20	89.5	14.2		20	75.7	19.5		20	75.6	7.3	
Cymiazole	5	66.7	9.1	0.9972	5	73.6	4.0	0.9875	5	90.9	9.5	0.9948
	10	71.6	7.3		10	83.8	12.4		10	79.0	23.4	
	20	84.5	7.9		20	75.8	15.3		20	85.7	21.9	
Derquantel	5	102.7	11.0	0.9963	5	107.0	3.2	0.9996	5	105.1	9.4	0.9991
	10	80.6	9.6		10	84.0	7.1		10	104.0	5.0	
	20	76.5	13.8		20	75.8	8.9		20	101.4	10.3	

Table 2. Validation of the analytical method for the 54 target compounds (n=5) (continued)

Compound	Beef				Pork				Chicken			
	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r²	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r²	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r²
Diaveridine	5	113.3	5.8	0.9993	5	98.8	9.7	0.9987	5	99.4	3.8	0.9944
	10	103.0	9.6		10	100.7	4.5		10	110.7	3.2	
	20	101.2	12.8		20	95.2	8.0		20	109.9	2.0	
Diethylcarbamazine	5	94.5	5.8	0.9988	5	94.5	13.1	0.9993	5	94.4	20.7	0.9992
	10	96.3	12.0		10	103.0	5.5		10	95.5	12.0	
	20	86.3	5.3		20	88.7	12.5		20	82.1	21.7	
Emamectin	5	93.3	3.0	0.9971	5	68.9	10.1	0.9843	5	104.5	13.1	0.9985
	10	101.5	13.8		10	74.8	11.3		10	95.7	7.8	
	20	106.6	5.6		20	86.0	13.6		20	84.2	18.9	
Febantel	50	87.3	11.3	0.9945	50	87.8	4.9	0.9982	50	93.4	17.5	0.9987
	100	76.6	8.2		100	88.2	9.4		100	93.7	2.5	
	200	74.9	8.9		200	77.5	5.6		200	92.2	8.8	
Fenbendazole	50	73.6	14.3	0.9955	50	91.7	13.9	0.9926	50	107.3	8.2	0.9955
	100	84.3	16.7		100	96.6	27.7		100	107.5	6.9	
	200	109.9	5.2		200	93.6	17.3		200	103.4	17.5	
Fluazuron	100	116.5	7.2	0.9888	5	97.7	30.7	0.9592	5	94.2	15.0	0.9839
	200	108.2	13.8		10	78.6	19.2		10	112.7	15.3	
	400	103.7	6.1		20	109.6	12.0		20	84.5	19.9	
Flubendazole	5	100.2	8.0	0.9973	5	94.8	19.6	0.9978	5	110.9	13.6	0.9987
	10	82.8	6.2		10	88.5	8.1		10	98.6	10.6	
	20	78.1	6.7		20	70.4	14.7		20	88.5	12.0	
2-Amino flubendazole	5	79.7	10.4	0.9944	5	93.9	9.4	0.9985	5	107.5	7.7	0.9972
	10	87.4	20.8		10	99.5	7.6		10	102.4	9.9	
	20	99.3	9.2		20	94.7	12.8		20	101.5	6.4	
Guaifenesin	5	111.1	29.5	0.9936	5	80.9	25.3	0.9884	5	82.9	20.8	0.9961
	10	102.9	20.3		10	86.0	20.0		10	103.3	16.2	
	20	101.8	13.1		20	83.7	13.9		20	108.6	15.1	
Halofuginone	5	84.9	13.6	0.9976	5	118.4	7.2	0.9973	5	113.2	8.6	0.9990
	10	90.3	13.5		10	89.6	10.5		10	97.2	6.9	
	20	81.4	6.5		20	71.4	4.0		20	95.3	15.3	
Imidocarb	150	99.2	11.9	0.9844	5	104.6	11.0	0.9983	5	103.9	17.0	0.9916
	300	88.8	15.6		10	106.0	9.0		10	103.4	17.3	
	600	91.5	9.2		20	97.5	15.5		20	80.4	16.1	

Table 2. Validation of the analytical method for the 54 target compounds (n=5) (continued)

Compound	Beef				Pork				Chicken			
	Target concentration levels (µg/kg)	Rec. (%)	CV (%)	r²	Target concentration levels (µg/kg)	Rec. (%)	CV (%)	r²	Target concentration levels (µg/kg)	Rec. (%)	CV (%)	r²
Isometamidium	50	114.5	7.7	0.9987	5	91.5	16.2	0.9883	5	83.4	12.1	0.9974
	100	106.5	14.8		10	74.2	19.6		10	65.5	8.4	
	200	95.4	9.5		20	76.4	19.0		20	78.4	18.1	
Levamisole	5	111.8	2.2	0.9978	5	102.4	5.6	0.9994	5	95.3	12.0	0.9994
	10	104.3	7.5		10	90.2	12.1		10	98.1	3.6	
	20	88.8	8.9		20	90.1	10.5		20	94.8	10.0	
Mebendazole	5	91.5	5.0	0.9995	30	91.3	5.4	0.9981	30	114.2	9.7	0.9995
	10	78.7	11.6		60	97.1	9.9		60	111.3	3.8	
	20	100.4	7.3		120	90.5	6.3		120	103.6	6.9	
Mebendazole amine	5	94.4	10.6	0.9802	30	84.3	8.0	0.9994	30	115.5	10.0	0.9993
	10	110.9	5.0		60	84.5	5.4		60	107.2	10.7	
	20	104.0	12.3		120	85.1	6.4		120	97.8	7.4	
5-Hydroxy mebendazole	5	93.8	5.8	0.9993	30	95.5	8.0	0.9991	30	100.3	8.3	0.9962
	10	84.6	8.3		60	89.0	7.1		60	103.8	6.8	
	20	78.5	19.2		120	83.1	4.8		120	102.6	4.9	
Methylbenzoquate	5	78.1	6.6	0.9860	5	99.9	14.7	0.9911	5	88.8	11.5	0.9942
	10	81.6	22.4		10	89.0	15.6		10	117.6	20.9	
	20	99.9	14.2		20	85.8	12.4		20	89.2	11.8	
Monensin	25	73.5	7.1	0.9966	25	84.3	9.9	0.9945	25	110.9	15.1	0.9916
	50	78.6	10.5		50	86.4	13.9		50	93.3	15.9	
	100	90.2	15.9		100	86.3	20.0		100	102.1	10.9	
Morantel	5	114.7	6.6	0.9985	5	81.9	10.6	0.9983	5	96.1	7.1	0.9960
	10	87.0	19.3		10	87.1	16.4		10	99.7	6.2	
	20	83.8	9.8		20	90.0	5.3		20	94.1	7.5	
Nicarbazin	5	77.3	3.0	0.9936	5	66.5	7.5	0.9876	5	106.1	7.4	0.9999
	10	77.6	9.3		10	74.4	15.0		10	106.9	6.7	
	20	90.6	10.2		20	83.6	12.2		20	98.6	9.6	
Niclosamide	5	107.1	11.2	0.9853	5	75.7	8.4	0.9899	5	106.3	19.9	0.9988
	10	87.8	25.8		10	87.8	31.5		10	98.7	15.2	
	20	84.1	13.9		20	93.2	18.4		20	106.0	20.5	
Ornidazole	5	89.4	13.4	0.9994	5	78.2	26.6	0.9958	5	91.2	16.7	0.9912
	10	79.6	15.0		10	108.6	9.0		10	101.9	11.0	
	20	89.5	11.0		20	106.3	10.1		20	105.0	14.4	

Table 2. Validation of the analytical method for the 54 target compounds (n=5) (continued)

Compound	Beef				Pork				Chicken			
	Target concentration levels (µg/kg)	Rec. (%)	CV (%)	r^2	Target concentration levels (µg/kg)	Rec. (%)	CV (%)	r^2	Target concentration levels (µg/kg)	Rec. (%)	CV (%)	r^2
Oxantel	5	93.4	4.7	0.9999	5	98.2	4.2	0.9997	5	99.5	6.0	0.9982
	10	91.9	7.0		10	99.2	2.0		10	107.3	1.8	
	20	94.2	7.4		20	95.8	3.3		20	107.2	2.2	
Oxfendazole	50	110.1	8.1	0.9998	50	87.9	9.3	0.9989	50	99.8	7.6	0.9974
	100	106.7	4.5		100	102.7	7.8		100	99.3	8.3	
	200	105.1	8.0		200	105.9	4.7		200	96.3	5.9	
Oxfendazole sulfone	50	99.3	2.3	0.9979	50	111.8	12.7	0.9992	50	104.3	6.9	0.9994
	100	96.3	4.0		100	100.1	9.1		100	102.7	9.4	
	200	103.8	5.1		200	101.6	7.8		200	100.8	6.1	
Oxibendazole	50	93.6	8.5	0.9942	50	84.6	9.1	0.9977	50	113.9	11.3	0.9994
	100	86.6	10.4		100	88.0	6.3		100	110.3	2.6	
	200	92.3	8.8		200	85.6	10.4		200	106.8	6.6	
Oxyclozanide	5	104.8	10.4	0.9970	5	93.0	9.4	0.9940	5	104.0	13.7	0.9959
	10	91.8	3.7		10	98.2	14.2		10	111.6	6.5	
	20	94.1	5.3		20	104.3	12.8		20	113.5	11.1	
Praziquantel	5	104.0	9.9	0.9992	5	91.8	9.4	0.9993	5	98.9	5.3	0.9953
	10	86.4	17.9		10	110.4	12.3		10	114.1	11.2	
	20	90.6	7.6		20	114.3	8.6		20	116.1	8.2	
Pyrantel	5	97.6	8.8	0.9989	5	111.8	9.1	0.9953	5	114.3	15.0	0.9995
	10	100.5	4.7		10	98.0	5.9		10	112.7	6.3	
	20	97.3	12.3		20	88.3	9.3		20	95.1	15.1	
Semduramicin	5	76.7	22.0	0.9930	5	80.8	25.6	0.9957	5	110.0	15.3	0.9974
	10	79.1	20.6		10	95.7	24.7		10	85.7	13.0	
	20	96.5	21.7		20	112.4	10.5		20	94.5	14.8	
Ternidazole	5	92.4	29.6	0.9807	5	91.4	22.9	0.9947	5	102.7	15.4	0.9843
	10	107.0	9.1		10	107.9	8.5		10	112.1	8.2	
	20	84.6	20.7		20	80.9	14.9		20	108.1	6.0	
Tetramisole	5	100.1	4.5	0.9997	5	90.8	3.1	0.9992	5	105.0	2.1	0.9999
	10	100.3	5.5		10	97.4	4.1		10	100.5	4.5	
	20	98.8	5.7		20	95.4	2.9		20	102.0	2.0	
Thiabendazole	5	101.6	7.6	0.9998	5	78.7	15.1	0.9966	5	101.4	10.4	0.9970
	10	103.3	3.0		10	110.3	5.1		10	119.9	4.0	
	20	106.6	5.5		20	114.2	7.4		20	116.8	7.8	

Table 2. Validation of the analytical method for the 54 target compounds (n=5) (continued)

Compound	Beef				Pork				Chicken			
	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r²	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r²	Target concentration levels (μg/kg)	Rec. (%)	CV (%)	r²
5-Hydroxy thiabendazole	5	110.9	18.3	0.9931	5	93.1	14.4	0.9950	5	116.7	18.0	0.9946
	10	90.7	11.4		10	116.9	7.2		10	105.6	18.7	
	20	76.4	9.9		20	111.8	7.9		20	72.0	17.7	
Thiophanate	5	117.4	9.3	0.9908	5	84.1	10.0	0.9974	5	109.9	15.8	0.9941
	10	84.1	16.2		10	103.9	20.9		10	92.4	4.2	
	20	90.2	4.2		20	93.4	4.8		20	94.3	10.9	
Tinidazole	5	78.7	10.6	0.9889	5	96.3	12.0	0.9997	5	94.6	17.1	0.9985
	10	103.3	9.0		10	93.0	9.3		10	86.2	9.6	
	20	100.9	9.3		20	73.7	9.2		20	92.5	6.9	
Toltrazuril sulfone	50	98.3	8.1	0.9954	50	86.0	11.8	0.9996	50	111.5	7.0	0.9992
	100	95.0	9.5		100	101.4	7.5		100	108.3	3.4	
	200	101.1	10.2		200	104.3	3.8		200	103.9	7.6	
Triclabendazole	100	85.3	15.5	0.9832	5	86.8	13.1	0.9983	5	110.5	7.8	0.9970
	200	92.4	8.1		10	83.0	15.5		10	109.5	12.3	
	400	107.1	12.2		20	82.6	19.7		20	104.8	6.6	
Keto triclabendazole	100	82.5	7.4	0.9929	5	91.2	8.1	0.9899	5	104.6	25.2	0.9951
	200	87.7	11.0		10	90.2	15.9		10	107.7	11.5	
	400	92.2	7.9		20	83.7	16.1		20	96.1	13.9	

Rec., recovery; CV, coefficient validation.

Matrix effects

The matrix effects observed for the various samples and compounds are presented in Fig. 1 and Table 4. Fig. 1 shown that the positive and negative matrix effects were observed for the livestock products examined herein. These effects were classified into five groups, namely high signal suppression (ME<−50%), moderate suppression (ME<−10% to −50%), no matrix effect (ME>−10% to <10%), moderate signal enhancement (ME>10% to <50%), and high signal enhancement (ME>50%; Chatterjee et al., 2016). It was found that the matrix effects varied in the range of −95%–56%, wherein high matrix effects were observed for 11 compounds (20.0%) in the beef matrix, nine compounds (16.4%) in the pork matrix, and seven compounds (12.7%) in the chicken matrix. No matrix effect was observed for 9 compounds (16.4%) in beef samples, 17 compounds (30.9%) in the pork samples, and 15 compounds (27.3%) in the chicken samples. In addition, moderate matrix effects were observed for 35 compounds (63.6%) in the beef matrix, 29 compounds (52.7%) in the pork matrix, and 33 compounds (60.0%) in the chicken matrix. Most compounds (beef, 47; pork, 40; chicken, 47) exhibited signal suppression, while a few (beef, 6; pork, 14; chicken, 7) exhibited signal enhancement. Pyrantel is neither in both suppression and signal enhancement in beef. The beef and chicken matrices were largely responsible for signal suppression, while the pork matrix led to both signal suppression and enhancement. The greatest suppression and enhancement were observed for the pork

Table 3. The LODs, LOQs (µg/kg) and Korean MRLs (µg/kg) for livestock products

Compounds	Beef			Pork			Chicken		
	MRL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	MRL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	MRL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Abamectin	10	0.03	0.1	10	0.1	0.3		0.03	0.1
Albendazole	100	0.03	0.1	100	0.03	0.1	100	0.03	0.1
Albendazole sulfone	100	0.1	0.3	100	0.1	0.3	100	0.1	0.3
Albendazole sulfoxide	100	0.2	0.6	100	0.3	1	100	0.2	0.7
2-Amino albendazole sulfone	100	0.1	0.2	100	0.1	0.4	100	0.1	0.4
Arprinocid		0.03	0.1		0.03	0.1		0.03	0.1
Benznidazole		0.2	0.7		0.2	0.5		0.2	0.6
Bithionol	10	0.3	1		0.3	0.9		0.1	0.2
Buquinolate		0.03	0.1		0.03	0.1		0.03	0.1
Cambendazole		0.1	0.2		0.1	0.2		0.1	0.2
Carbendazim		0.03	0.1	10	0.1	0.2		0.03	0.1
Carnidazole		0.03	0.1		0.03	0.1		0.03	0.1
Chlorfluazuron		0.1	0.2		0.03	0.1		0.03	0.1
Cymiazole		0.03	0.1		0.03	0.1		0.03	0.1
Derquantel		0.1	0.4		0.1	0.3		0.1	0.2
Diaveridine		0.03	0.1		0.03	0.1	50	0.1	0.3
Diethylcarbamazine	10	0.2	0.5		0.2	0.5		0.1	0.4
Emamectin		0.03	0.1		0.03	0.1		0.03	0.1
Febantel	100	0.03	0.1	100	0.03	0.1	50	0.03	0.1
Fenbendazole	100	0.1	0.3	100	0.1	0.2	50	0.1	0.2
Fluazuron	200	0.2	0.5		0.03	0.1		0.03	0.1
Flubendazole		0.03	0.1	10	0.03	0.1	200	0.03	0.1
2-Amino flubendazole		0.03	0.1	10	0.1	0.3	200	0.1	0.2
Guaifenesin	10	0.8	2.4	10	1.3	3.8	10	1	3
Halofuginone		0.03	0.1		0.03	0.1		0.03	0.1
Imidocarb	300	0.5	1.5		0.03	0.1		0.5	1.5
Isometamidium	100	0.1	0.2		0.03	0.1		0.03	0.1
Levamisole	10	0.03	0.1	10	0.03	0.1	10	0.03	0.1
Mebendazole		0.03	0.1	60	0.03	0.1	60	0.03	0.1
Mebendazole amine		0.1	0.3	60	0.1	0.4	60	0.2	0.6
5-Hydroxy mebendazole		0.2	0.5	60	0.1	0.3	60	0.2	0.5
Methylbenzoquate		0.03	0.1		0.03	0.1	10	0.03	0.1
Monensin	50	0.03	0.1	50	0.03	0.1	50	0.03	0.1
Morantel		0.9	2.6		0.9	2.7		1.1	3.4
Nicarbazin		0.1	0.2		0.03	0.1	200	0.1	0.2

Table 3. The LODs, LOQs (µg/kg) and Korean MRLs (µg/kg) for livestock products (continued)

Compounds	Beef			Pork			Chicken		
	MRL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	MRL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)	MRL (µg/kg)	LOD (µg/kg)	LOQ (µg/kg)
Niclosamide		0.2	0.5		0.1	0.3		0.1	0.2
Ornidazole		0.1	0.2		0.1	0.4		0.2	0.5
Oxantel		0.2	0.5		0.2	0.5		0.2	0.5
Oxfendazole	100	0.03	0.1	100	0.03	0.1	50	0.03	0.1
Oxfendazole sulfone	100	0.03	0.1	100	0.03	0.1	50	0.03	0.1
Oxibendazole	100	0.03	0.1	100	0.03	0.1		0.0	0.1
Oxyclozanide		0.2	0.7		0.2	0.5		0.1	0.4
Praziquantel		0.03	0.1		0.03	0.1		0.0	0.1
Pyrantel		3.2	9.7		0.2	0.5		0.2	0.6
Semduramicin		0.03	0.1		0.03	0.1	100	0.3	1
Ternidazole		0.1	0.2		0.1	0.3		0.1	0.3
Tetramisole	10	0.03	0.1	10	0.03	0.1	10	0.0	0.1
Thiabendazole	10	0.03	0.1	10	0.03	0.1		0.03	0.1
5-Hydroxy thiabendazole	10	0.1	0.2	10	0.03	0.1		0.03	0.1
Thiophanate		0.03	0.1	10	0.03	0.1		0.03	0.1
Tinidazole		0.03	0.1		0.1	0.2		0.03	0.1
Toltrazuril sulfone	100	0.1	0.3	100	0.03	0.1	100	0.03	0.1
Triclabendazole	200	0.03	0.1		0.03	0.1		0.03	0.1
Keto triclabendazole	200	0.3	0.9		0.1	0.2		0.1	0.2

No MRL values were set for the blanks.
LOD, limit of detection; LOQ, limit of quantification; MRL, maximum residue limit.
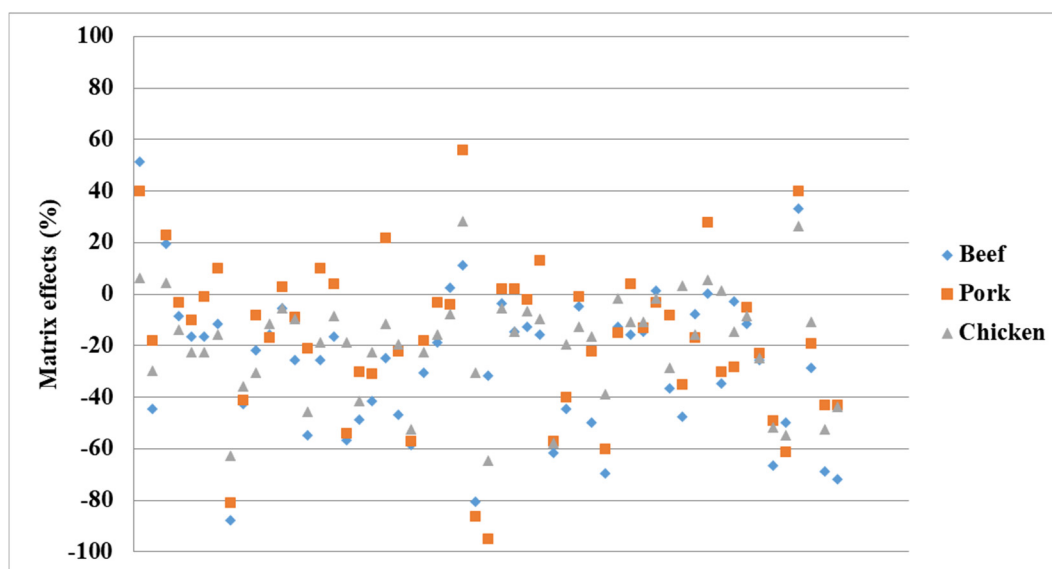
Fig. 1. Sample matrix effects of the target compounds in the beef, pork, and chicken matrices.

Table 4. Matrix effects of the 54 target compounds

No.	Compound	Beef	Pork	Chicken
1	Abamectin	51	40	6
2	Albendazole	−45	−18	−30
3	Albendazole sulfone	19	23	4
4	Albendazole sulfoxide	−9	−3	−14
5	2-Amino albendazole sulfone	−17	−10	−23
6	Arprinocid	−17	−1	−23
7	Benznidazole	−12	10	−16
8	Bithionol	−88	−81	−63
9	Buquinolate	−43	−41	−36
10	Cambendazole	−22	−8	−31
11	Carbendazim	−16	−17	−12
12	Carnidazole	−6	3	−6
13	Chlorfluazuron	−26	−9	−10
14	Cymiazole	−55	−21	−46
15	Derquantel	−26	10	−19
16	Diaveridine	−17	4	−9
17	Diethylcarbamazine	−49	−30	−42
18	Emamectin	−42	−31	−23
19	Febantel	−25	22	−12
20	Fenbendazole	−47	−22	−20
21	Fluazuron	−59	−57	−53
22	Flubendazole	−31	−18	−23
23	2-Amino flubendazole	−19	−3	−16
24	Guaifenesin	2	−4	−8
25	Halofuginone	11	56	28
26	Imidocarb	−81	−86	−31
27	Isometamidium	−32	−95	−65
28	Levamisole	−4	2	−6
29	Mebendazole	−15	2	−15
30	Mebendazole amine	−13	−2	−7
31	5-Hydroxy mebendazole	−16	13	−10
32	Methylbenzoquate	−62	−57	−58
33	Monensin	−45	−40	−20
34	Morantel	−5	−1	−13
35	Nicarbazin	−50	−22	−17
36	Niclosamide	−70	−60	−39
37	Ornidazole	−13	−15	−2
38	Oxantel	−16	4	−11

Table 4. Matrix effects of the 54 target compounds (continued)

No.	Compound	Beef	Pork	Chicken
39	Oxfendazole	−15	−13	−11
40	Oxfendazole sulfone	1	−3	−2
41	Oxibendazole	−37	−8	−29
42	Oxyclozanide	−48	−35	3
43	Praziquantel	−8	−17	−16
44	Pyrantel	0	28	5
45	Semduramicin	−35	−30	1
46	Ternidazole	−3	−28	−15
47	Tetramisole	−12	−5	−9
48	Thiabendazole	−26	−23	−25
49	5-Hydroxy thiabendazole	−67	−49	−52
50	Thiophanate	−50	−61	−55
51	Tinidazole	33	40	26
52	Toltrazuril sulfone	−29	−19	−11
53	Triclabendazole	−69	−43	−53
54	Keto triclabendazole	−72	−43	−44

matrix, and these corresponded to −95% and 56% for isometamidium and halofuginone, respectively. These variable matrix effects are likely due to the complexity of the tissue matrix. Although the most effective means to compensate for matrix effects is to use an internal standard (Yin et al., 2016), internal standards are expensive, and the corresponding compounds for the various target compounds are often unavailable. Thus, the current study was performed using a matrix-matched standard curve.

Application of our method to real samples

To demonstrate the applicability of our method, the livestock samples (n=30) collected from Korean local markets were analyzed. Among these samples, toltrazuril sulfone was detected in pork and chicken samples at concentrations of 1 μg/kg in pork (2 samples) and 5 μg/kg in chicken (1 sample); however, it should be noted that their concentrations were lower than the Korean MRL (Table 3). Toltrazuril is a triazine-based antiprotozoal that is commonly used in pigs and chicken turkeys (Mehlhorn et al., 1988). Although toltrazuril sulfone is reportedly more effective in smaller amounts than toltrazuril, it is highly toxic and can cause side effects if consumed by humans through the food chain (Franklin et al., 2003; Lindsay et al., 2000).

In a previous study, toltrazuril and toltrazuril sulfone were detected in frankfurter sausages at a concentration of 2 μg/kg (Martínez-Villalba et al., 2010). Indeed, the detection of anthelmintic and antiprotozoal drugs in livestock samples has been widely reported (Adesiyun et al., 2021; Pawar et al., 2021; Yoo et al., 2021). Ai et al. (2011) detected diclazuril in rabbit muscles (n=10), while monensin (1.4–22 ng/g, n=42) and ractopamine (0.6–64 ng/g, n=15) were detected in bovine liver, and monensin (0.8 and 1.1 ng/g, n=2) and ractopamine (1.8–6.3 ng/g, n=12) were detected in bovine muscle. Ractopamine (0.5–67 ng/g, n=7) was detected in bovine kidney, while monensin (2.0 ng/g, n=1), decoquinate (150 ng/g, n=1), lasalocid (1.5 and

14 ng/g, n=2), narasin (4 ng/g, n=1), and *N,N′*-bis(4-nitrophenylurea; 190 ng/g, n=1) were detected in chicken muscle (Matus and Boison, 2016). Furthermore, according to Kang et al. (2015), acetyl salicylic acid (12–576 µg/kg; n=28, 50–53 µg/kg; n=1) was detected in pigs and chickens, paracetamol (28–381 µg/kg, n=15) was detected in pigs, clopidol (9–4,614 µg/kg) was detected in chickens (n=28) and ducks (n=6), while diclazuril and amprolium were detected in chicken livers (104–525 µg/kg, n=8; and 195–196 µg/kg, n=2, respectively). Moreover, toltrazuril and its metabolites (toltrazuril sulphone and toltrazuril sulfoxide) were detected in chicken liver (n=29) at concentrations of 161–469, 67–1,822, and 209–760 µg/kg, respectively, while phenylbutazone and its metabolite (oxyphenylbutazone) were detected at levels of 247 and 15 µg/kg in cattle liver (n=1), respectively, and nicarbazin was detected at a concentration of 0.05 µg/kg in eggs (n=1; Kang et al., 2015).

Overall, this study shows that the detection amount is smaller than in previous studies (Ai et al., 2011; Matus and Boison, 2016). Therefore, monitoring results shows that livestock products are a safe level of residues. Therefore, the UHPLC-MS/MS method established in this study can be used as a reliable method for the detection of anthelmintic and antiprotozoal drug residues.

Conclusion

We herein reported the validation of an analytical method for the simultaneous quantification of anthelmintic and antiprotozoal drugs in livestock products (i.e., beef, pork, and chicken). This method exhibited an overall satisfactory performance in terms of its accuracy and precision, thereby indicating its applicability as a quantitative method. In addition, the current method achieved low limits of quantitation (0.1–9.7 µg/kg) for all target compounds in the beef, pork, and chicken. Following the successful analysis of 30 real samples obtained from markets in Korea, three samples gave detection rate of 10%; however, the residual concentrations did not exceed those of the Korean MRLs. Thus, the obtained the results confirm the suitability of this method for the detection of anthelmintic and antiprotozoal drugs in livestock products. Further study needs to increase the number of real samples and have to perform a risk assessment for the detected results. In addition, previous studies show the detection of residues in by-products (Kang et al., 2015). Therefore, it is necessary to conduct extended experiments on by-products. Nevertheless, the proposed method can be used to successfully perform the routine analysis of residues in livestock products, thereby significantly contributing to the development of multi-residue analysis and safety management in the future. Also, we expect to use the developed method to prepare for PLS program for veterinary drug in livestock and fishery products produced in 2024.

Supplementary Materials

Supplementary materials are only available online from: https://doi.org/10.5851/kosfa.2023.e41.

Conflicts of Interest

The authors declare no potential conflicts of interest.

Acknowledgements

Author Contributions

Conceptualization: Choi JD, Moon G. Methodology: Kim E. Validation: Park H, Lee TH, Park S. Investigation: Park H. Writing - original draft: Park H. Writing - review & editing: Park H, Kim E, Lee TH, Park S, Choi JD, Moon G.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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