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Validation of a new screening, determinative, and confirmatory multi-residue method for nitroimidazoles and their hydroxy metabolites in turkey muscle tissue by liquid chromatography-tandem mass spectrometry

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A new and sensitive multi-residue method (MRM) with detection by LC-MS/MS was developed and validated for the screening, determination, and confirmation of residues of 7 nitroimidazoles and 3 of their metabolites in turkey muscle tissues at concentrations ≥ 0.05 ng/g. The compounds were extracted into a solvent with an alkali salt. Sample clean-up and concentration was then done by solid-phase extraction (SPE) and the compounds were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The characteristic parameters including repeatability, selectivity, ruggedness, stability, level of quantification, and level of confirmation for the new method were determined. Method validation was achieved by independent verification of the parameters measured during method characterization. The seven nitroimidazoles included are metronidazole (MTZ), ronidazole (RNZ), dimetridazole (DMZ), tinidazole (TNZ), ornidazole (ONZ), ipronidazole (IPR), and carnidazole (CNZ).

It was discovered during the single laboratory validation of the method that five of the seven nitroimidazoles (i.e. metronidazole, dimetridazole, tinidazole, ornidazole and ipronidazole) and the 3 metabolites (1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (MTZ-OH), 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI, the common metabolite of ronidazole and dimetridazole), and 1-methyl-2-(2′-hydroxyisopropyl)-5-nitroimidazole (IPR-OH) included in this study could be detected, confirmed, and quantified accurately whereas RNZ and CNZ could only be detected and confirmed but not accurately quantified.

Keywords: performance characteristics; multi-residue method; nitroimidazoles; metabolites; LC-tandem mass spectrometry; turkey tissues

Introduction

A new and sensitive multi-residue method (MRM) with detection by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed for the determination and confirmation of residues of 7 nitroimidazoles and 3 of their metabolites in poultry muscle, porcine muscle, kidney, and liver (Figure 1). The 5-nitroimidazoles are banned for use by Health Canada[1] in food-producing animals as they are suspected to be carcinogenic and mutagenic.[2]

Methods have been published for the determination of nitroimidazole compounds which require one[3–6] or two[7–10] evaporation steps. During the method development process, the evaporation step was identified as a critical step which resulted in inconsistent recoveries; this step was also identified as a critical point in a previously published method.[3] Therefore, this method was developed to avoid all evaporation steps. It also utilizes LC-MS/MS which provides quantification and confirmation data, and there is no need for derivatization as required for analysis by GC-MS.[6]

Performance characteristic parameters, factors which in practice demonstrate how well a method performs and include the working range, precision, recovery, potential interferences, accuracy, selectivity, and a measure of the uncertainty of the method, were determined for the method. The ultimate objective of this method development and characterization project was to use this method for regulatory purposes. Therefore, the operational parameters included those listed above, which are generally considered appropriate in guidance documents prepared by international agencies.[11,12]

This paper presents the experiments that were conducted to measure the characteristic performance parameters for the newly developed method and the results of the validation study conducted to determine whether the method meets the requirements for use in a regulatory monitoring program for veterinary drug residues in turkey muscle tissues.

Experimental

Chemicals and reagents

Ethyl acetate, methanol, acetonitrile, and hexane, all high purity grade, were purchased from Caledon (Georgetown, ON, Canada). Formic acid (minimum 98%), and ammonium hydroxide (28–30% was obtained from VWR (Mississauga, ON, Canada).

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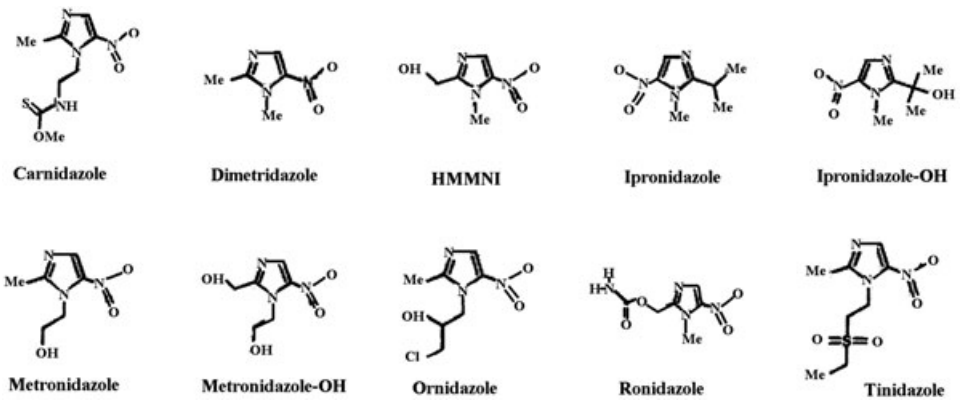

Figure 1. Chemical structure of the nitroimidazoles and their hydroxy metabolites.

Table 1. Gradient condition for the LC-MS/MS analysis of the nitroimidazoles

Time (min.)	Flow rate (mL/min.)	Methol	0.10% Formic Acid	Curve
0	0.25	40	60	-
5.5	0.25	70	30	6
10.5	0.25	98	2	1
12.5	0.35	99	1	1
12.8	0.25	40	60	6
15	0.25	40	60	6

Individual stock standard solutions (100–200 μg/ml) of each of the drugs of interest (obtained from Sigma-Aldrich Canada Ltd. Oakville, Ontario, Canada, Fitzgerald Acton, MA, USA, EQ Laboratories, Augsburg, Germany) were prepared by dissolving between 0.00500 g and 0.01000 g in methanol in 50-ml volumetric flasks. A mixed intermediate standard at a concentration of 2.00μg/ml was prepared from which a mixed working standard solution at a concentration of 25 ng/ml was prepared.

Instrumentation

A Thermo Hypercarb analytical column was used, 2.1 x 50mm 3 μm at 50°C. A Waters Acquity UPLC interfaced to a Waters Micromass

Table 2. Ruggedness experimental design and results for turkey muscle tissue

Step in procedure	Activity			Step in procedure		Change in activity		
A	Add 12 ml ethyl acetate			a		Add 10 ml ethyl acetate		
B	Add 0.5 ml Na_2CO_3			b		Add 0.625 ml Na_2CO_3		
C	Add 0.5 ml NaCl			c		Add 0.625 ml NaCl		
D	Add 500 μL formic acid			d		Add 450 μL formic acid		
E	Wash with 2 ml 2% formic acid in Ethyl acetate			e		Wash with 1.5 ml 2% formic acid in Ethyl acetate		
F	Wash with 6 ml acetonitrile			f		Wash with 6.5 ml acetonitrile		
G	Dry SPEs for 10 min			g		Dry SPEs for 25 min		
Experiment								
	1	2	3	4	5	6	7	8
A/a	A	A	A	A	a	a	a	a
B/b	B	B	b	B	B	b	b	b
C/c	C	c	C	c	C	c	C	c
D/d	D	D	d	d	d	d	D	D
E/e	E	e	E	e	e	E	e	E
F/f	F	f	f	F	F	f	f	F
G/g	G	g	g	G	g	G	G	g
Experimental results obtained from ruggedness study on negative control samples fortified with the drugs of interest								
	Observed Experimental Results (% Recovery)							
MTZ-OH	101	108	107	106	95	97	87	92
RNZ	110	107	98	95	98	101	125	105
MTZ	105	105	110	107	97	99	100	95
HMMNI	100	106	108	101	99	101	95	98
DMZ	104	104	107	99	97	104	93	99
TNZ	101	107	105	101	99	100	93	93
ONZ	101	107	108	96	99	101	93	96
IPR-OH	105	104	113	103	102	102	97	97
IPR	101	109	107	99	100	100	96	95
CNZ	98	97	96	95	92	95	77	99

Table 3. Linear regression parameters for the calibration function for turkey muscle (Breast)

Nitroimidazole	Analytical Range (ng/g)	LOQ (ng/g)	Slope of Linear Regression Line	Intercept of Linear Regression Line	Coefficient of Variation (r)
Metronidazole-OH	0.05 – 0.50	0.05	4666	−341	0.997
Ronidazole	0.05 – 0.50	0.05	16201	−771	0.997
Metronidazole	0.05 – 0.50	0.05	42132	−2568	0.996
HMMNI	0.05 – 0.50	0.05	16284	−874	0.998
Dimetridazole	0.05 – 0.50	0.05	57694	−2943	0.998
Tinidazole	0.05 – 0.50	0.05	21051	−995	0.998
Ornidazole	0.05 – 0.50	0.05	41597	−1318	0.999
Ipronidazole-OH	0.05 – 0.50	0.05	60476	−4390	0.993
Ipronidazole	0.05 – 0.50	0.05	38672	−1925	0.999
Carnidazole	0.10 – 0.50	0.10	23451	−4442	0.990

Table 4. MS/MS acquisition parameters for selected reaction monitoring (SRM) experiments for confirmation at the defined collision energy (CE) in eV

			Product Transition Ions								
	Precursor Ion (m/z)	Cone voltage	Quantification Ion (Q)		Q1		Q2		Q3		Dwell Time
Compound	(m/z)	(V)	Product 1	CE	Product 2	CE	Product 3	CE	Product 4	CE	Sec
MTZ-OH	188	25	125.9	15	122.9	15	143.9	15	67.9	20	0.25
RNZ	201	17	139.8	10	110	20	55	20			0.10
MTZ	172	23	127.8	15	81.8	30	55.9	20	111	20	0.10
HMMNI	158	23	139.8	10	54.9	20	94	20	112	20	0.15
DMZ	142	30	96	15	80.9	22	55	22			0.17
TNZ	248	27	120.8	15	127.9	20	81.8	30	92.7	20	0.15
ONZ	220	25	127.8	20	81.8	30	57	25	93	25	0.15
IPR-OH	186	25	167.9	15	121.7	20	127.8	15	81.8	20	0.10
IPR	170	30	109.9	20	123.9	15	95.8	20	84	20	0.12
CNZ	245	15	118	15	75	30					0.50

Q – Product Ion used for quantitative analysis.

Table 5. Retention time, ion ratios and tolerances measured for nitroimidazoles in fortified turkey muscle tissue for confirmatory analyses

Analyte		Ion Ratios for Quantitative Analyses		
	Retention time	Q1	Q2	Q3
	(min)	Ion ratio ± S.D.	Ion ratio ± S.D.	Ion ratio ± S.D.
MTZ-OH	3.51 ± 0.01	1.1 ± 0.1	3.0 ± 0.2	3.2 ± 0.2
MTZ	4.92 ± 0.02	3.0 ± 0.1	14.7 ± 1.9	28.5 ± 5.3
HMMNI	4.45 ± 0.01	2.5 ± 0.2	9.0 ± 0.9	10.7 ± 2.2
DMZ	5.84 ± 0.01	4.8 ± 0.4	12.6 ± 1.4	
TNZ	7.32 ± 0.01	3.3 ± 0.1	3.4 ± 0.2	36.1 ± 0.4
ONZ	7.64 ± 0.01	1.5 ± 0.05	7.0 ± 0.4	32.6 ± 3.5
IPR-OH	5.15 ± 0.01	2.3 ± 0.1	11.3 ± 0.9	6.1 ± 0.4
IPR	5.96 ± 0.01	0.7 ± 0.03	4.6 ± 0.3	18.6 ± 3.2
RNZ	4.95 ± 0.01	30.0 ± 8.4	3.0 ± 0.7	
CNZ	9.00 ± 0.01	6.5 ± 1.1		

Q = Quantification Ion from Table 3.

tandem quadrupole (Quattro Premier) mass spectrometer with a Z-spray interface, controlled by MassLynx software version 4.1 and operated under positive electrospray ionization (ESI+) mode was used for the separation and analysis of the samples. The gradient conditions listed in Table 1 were used to separate the components of the tissue extract in order to be chromatographically fed into the

mass spectrometer that was used for the detection and confirmation of the analytes. The MS was set up as follows: capillary voltage 2.7 kV, source temperature 110 °C, desolvation temperature 350 °C, cone gas (nitrogen) flow 50 L/h, desolvation gas flow 1100 L/h, collision gas (argon) flow 0.25 ml/min, and the mass spectrometer was set to one mass unit resolution.

Sample extraction and clean-up

Blank tissue and test tissue samples of 5.00 ± 0.05 g were weighed into individual 50-ml polypropylene centrifuge tubes. Appropriate volumes of the 25 ng/ml working standard solution were added to five blank turkey muscle tissues to prepare matrix fortified calibration standards equivalent to 0.05, 0.08, 0.10, 0.20, 0.50 ng/g, respectively. A sixth blank turkey muscle tissue was not fortified and served as a negative control sample.

Ten millilitres (ml) of ethyl acetate was added to each of the sample tubes and homogenized, and the homogenizer probe was rinsed with 2 ml of ethyl acetate into the sample tube. The homogenizer probe was cleaned between samples with ethyl acetate. Note that water should not be used to clean the homogenizer probe as this can decrease recoveries. After homogenization of

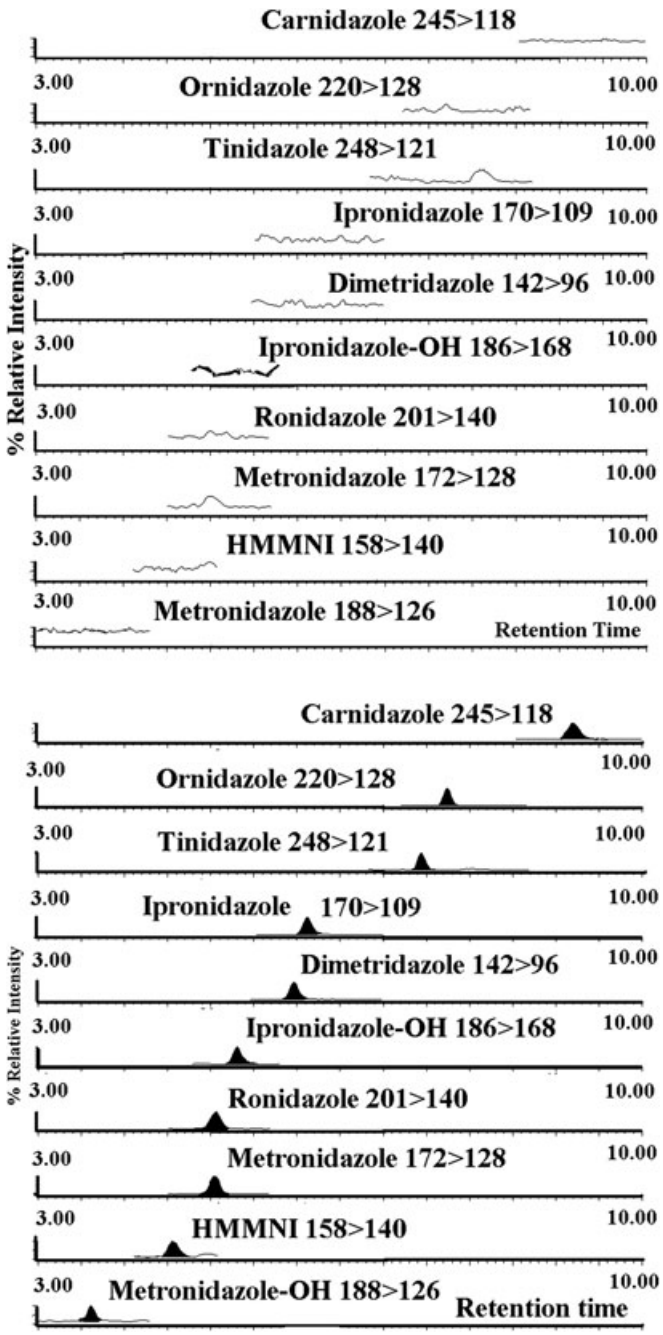

Figure 2. (A) A typical chromatogram of the quantification ion of an extract obtained from a turkey muscle blank tissue. (B) A typical chromatogram of the quantification ion of an extract obtained from a turkey muscle blank tissue fortified with the nitroimidazoles and the metabolites at a concentration of 0.05 ng/g each.

the samples, ~0.7–0.8 g (measured with a 0.5 ml spatula) of sodium chloride and ~ 0.7–0.8 g (measured with a 0.5-ml spatula) of anhydrous sodium carbonate was added to each sample tube. Samples were shaken for 10 min and centrifuged at 3900 g (approximately 4350 rpm) for 10 min at room temperature. The ethyl acetate was transferred to a clean 50-ml polypropylene tube, avoiding the transfer of any particulates or aqueous matter with the ethyl acetate. The tissue pellet was re-extracted with a second 10-ml aliquot of ethyl acetate, which was combined with the first extract. To the combined ethyl acetate extractions, 0.50 ml of formic acid was added and the solution was vortexed for 5–10 s. A Waters Oasis MCX cartridge (3 ml, 60 mg) was conditioned consecutively with 3 ml ethyl acetate and 3 ml 2% formic acid in ethyl acetate. To the top of the bed of the cartridge, ~0.05–0.06 g (measured with a 0.10-ml spatula) of anhydrous sodium sulphate was added, then ~0.05–0.06 g (measured with a 0.10-ml spatula) of granular carbon (mesh 20–40). The extract was loaded onto the MCX cartridge, ensuring that the carbon and sodium sulfate were saturated with the ethyl acetate extract. If a reservoir was used to load the sample it should be removed before washing the cartridges. After sample loading was complete, the cartridges were washed consecutively with 2 ml of 2% formic acid in ethyl acetate, 3 ml ethyl acetate, 3 ml hexane, 3 ml ethyl acetate, and twice with 3 ml acetonitrile. The cartridges were dried under vacuum at approximately −50 kPa (−15 inches Hg) for 5 min, the carbon and sodium sulfate were discarded, and the cartridges were dried under vacuum for an additional 15 min. The samples were eluted with 700 µl of 90/8/2 methanol/water/ammonium hydroxide into a tube containing 20 µl of formic acid. The solution was allowed to remain on the cartridge for 5–10 min, and the remaining eluate was drawn through by applying light vacuum for 30–60 s. The final volume of the extract was about 500 µl. The solution was vortexed for 5–10 s, and filtered with a Whatman™ 0.2 µm PTFE Mini-UniPrep™ syringeless filter vial, and 5 µl was injected on the LC-MS/MS system for analysis.

Results of the experiments to measure the characteristic operational parameters for the method

Ruggedness

Once the method was optimized, the Youden approach (Table 2), a fractional factorial design of 8 combinations of 7 variables was used to assess how rugged it was and also to determine if there were any critical control points in the method procedure that needed to be identified. Analysis and interpretation of the results of the ruggedness experiment conducted indicate that minor changes in the method allowing for changes of up to 10% did not have any significant effects on the analytical results. In addition, no critical control points were identified in the procedure.

Stability

Analyte stability in standard solution, in turkey muscle tissue extracts and stored tissue was tested over 16 weeks using the optimized method. A slight decrease in concentration for carnidazole was observed from weeks 5 to 6, while the other compounds were observed to be stable for up to 7 weeks. Hence, it was determined that turkey muscle tissue samples can be stored for up to 5 weeks at −20°C prior to analysis. A freeze/thaw stability study was also conducted in which the samples were thawed and frozen again in the first and second weeks, then thawed and analyzed in the fourth week. No significant change in concentration was observed due to the freeze/thaw cycles.

Analytical range and linearity

The analytical range for the method was 0.05 ng/g to 0.50 ng/g for each analyte except for carnidazole which had a range of 0.1 ng/g to 0.5 ng/g. For the five-point (plus blank) matrix fortified calibration curves whose linear regression parameters (without

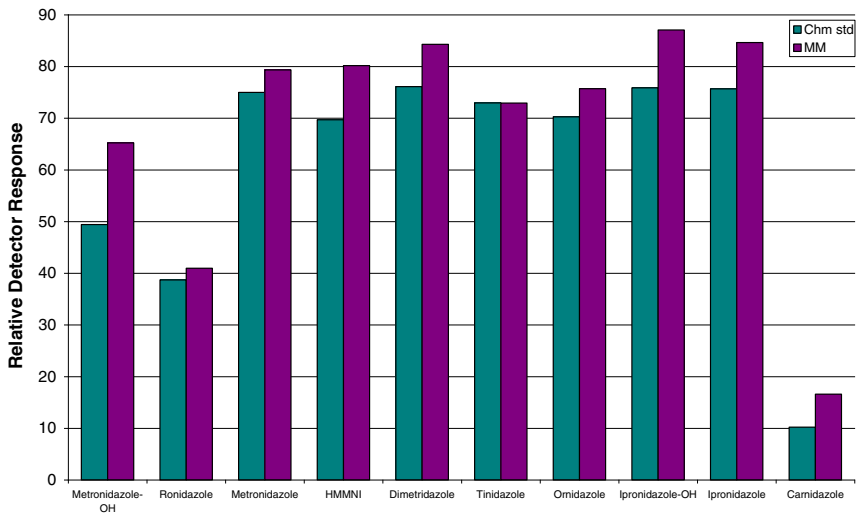

Figure 3. Estimation of the matrix effect resulting from the presence of turkey muscle tissue on the detector signal for the seven nitroimidazoles and their hydroxy metabolites.

Table 6. Intra-day precision (%RSD) and accuracy (bias) data generated by 2 independent analysts at 0.20, 0.30 and 0.40 ng/g of nitroimidazoles and their hydroxy metabolites fortified to drug-free turkey muscle tissues using the described method (n = 9 for each analyst)

Expanded MU (%) →	MTZ-OH			MTZ			HMMNI			DMZ			TNZ			ONZ			IPR-OH			IPR			RNZ			CNZ		
	14			23			21			15			19			16			20			15			25			26		
Concentration (ng/g) Analyst	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias	Mean	% RSD	% Bias
0.20 ng/g Analyst A	0.20	5	0	0.19	5	5	0.20	5	0	0.20	5	0	0.18	6	10	0.19	5	5	0.19	5	5	0.20	10	0	0.18	16	−10	0.22	16	+10
0.20 ng/g Analyst B	0.21	10	+5	0.20	5	0	0.19	10	−5	0.27	4	+35	0.18	6	−10	0.19	5	−5	0.20	5	0	0.20	5	0	0.21	22	+5	0.18	22	−9
0.30 ng/g Analyst A	0.29	7	−3	0.29	3	−3	0.30	7	0	0.29	3	−3	0.27	7	−10	0.28	4	−7	0.30	7	0	0.30	3	0	0.28	17	−7	0.29	8	−3
0.30 ng/g Analyst B	0.32	7	+7	0.32	6	+7	0.31	6	+3	0.31	6	+3	0.26	7	−13	0.29	7	−3	0.30	7	0	0.30	3	0	0.32	26	+8	0.27	12	−10
0.40 ng/g Analyst A	0.38	3	−5	0.39	3	−3	0.39	5	−3	0.39	3	−3	0.37	5	−8	0.39	3	−3	0.40	3	0	0.40	0	0	0.40	9	0	0.37	13	−8
0.40 ng/g Analyst B	0.40	5	0	0.38	3	−5	0.35	9	−13	0.38	5	−5	0.32	6	−20	0.37	5	−8	0.36	5	−10	0.37	3	−8	0.35	22	−12	0.38	26	−5

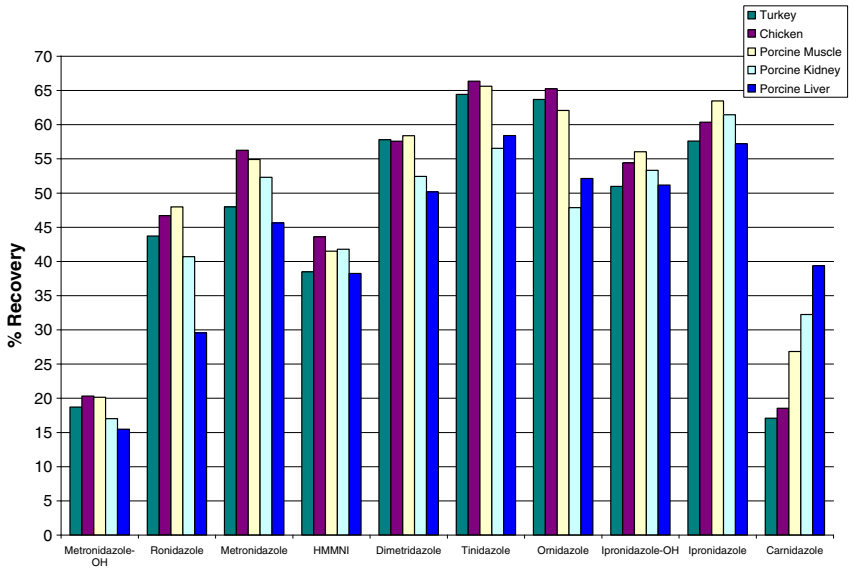

Figure 4. Graphical representation of recoveries achieved using the method without modification for the analysis of the nitroimidazoles and their metabolites in fresh turkey muscle tissue, chicken muscle tissue, and porcine muscle, kidney and liver tissues.

weighting factors) are shown in Table 3, the correlation coefficients (r) were typically ≥ 0.990.

Limit of confirmation (LOC) and Limit of quantification (LOQ)

The presence of the compound was confirmed when the following criteria were met: (1) the precursor ion, together with a minimum of two product transition ions were present (Table 4) and the ion ratios were within specified limits (Table 5); (2) the signal to noise of each ion was >3; and (3) the retention time of the compound in the sample matched that of the matrix fortified sample to within ± 2.5 %. The limits of confirmation ranged from 0.02 to 0.05 ng/g determined empirically. The limits of quantification (LOQ) were determined statistically to be 0.05 ng/g for all the nitroimidazoles and their hydroxy metabolites, except for carnidazole which was calculated to be 0.1 ng/g (Table 3) using the Minitab Statistical

Table 7. Results of the analysis of samples prepared by the Quality Manager (QM) and presented blind to an experienced analyst for analysis using the described method showing the fortified concentrations, the concentrations experimentally found using the method and the accuracy of the method for the quantification and identification of the nitroimidazoles in 'unknown' samples

	MTZ-OH			MTZ			HMMNI			DMZ		
Expanded MU (%)	14			23			21			15		
Sample ID	Spike	Found	Bias	Spike	Found	Bias (%)	Spike	Found	Bias (%)	Spike	Found	Bias (%)
41	0.28	0.27	−4	0.28	0.30	+7	0.28	0.33	+18	0.28	0.29	+4
46	0.28	0.27	−4	0.28	0.27	−4	0.28	0.28	0	0.28	0.29	+4
42				0.21	0.23	+10						
47				0.21	0.19	−10						
43	0.43	0.42	−2							0.43	0.41	−5
48	0.43	0.42	−2							0.43	0.45	+5
44							0.34	0.36	+6			
49							0.34	0.34	0			
45												
50												
% **False Positives**	**0**	**0**			**0**			**0**			**0**	
% **False Negatives**	**0**	**0**			**0**			**0**			**0**	
% **Accurately Quantified**		**100**			**100**			**100**			**100**	

Software (Release 12) package. These values were rounded up to be at or above the level of confirmation.

Figure 2 shows a typical chromatogram of the quantification ion of an extract obtained from a turkey muscle blank tissue (2A) and a turkey muscle blank tissue fortified with the nitroimidazoles and the metabolites at the limit of quantification of 0.05 ng/g for each analyte (2B). Figure 2A shows that there were no interfering components eluting and/or co-eluting with the nitroimidazoles and their hydroxy metabolites to interfere with the anticipated non-detectable signal to cause a false negative result. Additionally, Figure 2B shows that the method is able to detect all the nitroimidazoles and their hydroxy metabolites when they are intentionally added to tissue matrix at a concentration equal to the experimentally determined LOQ.

Matrix effect

The signals generated for each nitroimidazole at a defined concentration in neat standard solution were matched equally by those generated from the equivalent turkey muscle tissue matrix matched standards indicating that for this method, there was little or no matrix effect associated with the sample procedure and analysis (Figure 3). The matrix matched standards were prepared by extracting an equivalent blank sample according to the described procedure and adding the same concentration of nitroimidazole in the neat standard to the extract just prior to re-constituting for analysis.

Selectivity

To demonstrate whether the method would be capable of detecting truly negative turkey muscle tissue samples as negative with 95% confidence at a 5% prevalence rate, 60 blank turkey muscle tissues from 6 different tissue pools were analyzed with the method. All the results were negative and there were no false positive results. To determine whether other potentially interfering drugs such as thyreostats, fluoroquinolones, sulfonamides, and glycopeptides, if present as a residue in the tissue of a treated animal, could directly interfere with the anticipated test response from the method, drug-free turkey muscle tissue samples were fortified with compounds from each drug group and analyzed. None of these groups of compounds interfered with the anticipated negative response of the method. In other words, there were no false positive results. In addition, drug-free turkey muscle tissue samples were fortified at a defined concentration with each of the nitroimidazoles and the metabolites together with each of the groups of potentially interfering drugs and the samples were processed and analysed as before. It was determined that, if present, sulphonamides will suppress the signal response of ronidazole by 46 % and thyreostats will enhance the signal response for carnidazole by 90 %.

Precision (RSD), measurement uncertainty (MU) and accuracy (bias)

To evaluate intra-day precision and accuracy, 20 replicate samples at 3 different concentrations (0.05, 0.075, and 0.10 ng/g corresponding to 0.5 x LOQ, 1.5 x LOQ and 2.0 x LOQ, respectively) for a total of 60 turkey muscle tissue samples were extracted and analyzed along with a five point calibration curve in one day. To evaluate inter-day precision and accuracy, the intra-day experiment was repeated on two additional days. The intra-day and inter-day precision were both ≤15 % RSD. The accuracy of the method for each compound was calculated by comparing the experimentally determined concentration with the concentration added to the sample and expressed as a percentage (positive or negative bias). The intra-day accuracy ranged from −10 to +8 %, and the inter-day accuracy ranged from −8 to +5 %.

Table 7. (Continued)

TNZ			ONZ			IPR-OH			IPR			RNZ			CNZ	
19			16			20			15			25			26	
spike	Found	Bias (%)	Spike	Found	Bias (%)	Spike	Found	Bias (%)	Spike	Found	Bias (%)	Spike	Found	Bias (%)	Spike	Found
0.28	0.28	0	0.28	0.29	+4	0.28	0.30	+7	0.28	0.30	+7	0.28	0.22	−21	0.28	0.40
0.28	0.26	−7	0.28	0.25	−11	0.28	0.29	+4	0.28	0.27	−4	0.28	0.20	−29	0.28	0.43
0.21	0.20	−5							0.21	0.20	−5					
0.21	0.20	−5							0.21	0.19	−10					
															0.43	1.07
															0.43	0.76
0.46	0.43	−7				0.34	0.30	−12	0.34	0.32	−6	0.43	0.14	−67		
0.46	0.46	0				0.34	0.31	−9	0.34	0.35	+3	0.43	0.16	−63		
															0.15	0.34
															0.15	0.17
	0			**0**			**0**			**0**			**0**			**0**
	0			**0**			**0**			**0**			**0**			**0**
	100			**100**			**100**			**100**			**0**			**16**

The measurement uncertainty (MU) was calculated as the relative combined uncertainty (RU) for precision and recovery using the data generated for recovery and precision above. The expanded relative uncertainty, Expanded MU = k x RU, where k = 2 was ≤ 30 % for all the analytes (Table 6).

Method applicability

In addition to turkey muscle tissues, the method without any modification, was shown to be applicable to the analysis of the nitroimidazoles and their metabolites in fresh slaughter porcine (muscle, kidney, and liver) and chicken muscle tissues (Figure 4).

Single laboratory validation of the method

Once the characteristic operational parameters for the optimized method had been measured, it was subjected to a single laboratory validation in turkey muscle tissue. The validation experiments conducted by an independent analyst consisted of verifying the selectivity, accuracy, and precision claims of the method. The selectivity experiment consisted of testing six drug-free samples using the analytical method. The accuracy and precision characteristics of the method were verified by conducting replicate analysis at three concentrations around the LOQ of the method on three different days and comparing the results obtained with those obtained at characterization. Since there were no incurred residues or standard reference materials containing these compounds, samples were also prepared by the Quality Manager and provided blind to an experienced analyst for analyses.

Results of the single laboratory validation study

Results of the selectivity test showed that all test samples were confirmed as not containing any of the residues of the drugs of interest. The conclusion was that the selectivity claim for the method was verified and the author's claim that the method is selective was substantiated.

Table 6 compares the intra-day accuracy and precision data generated at characterisation with those generated under validation by an independent analyst. It is clear that the two data sets are similar and it can be concluded that there is no statistically significant difference between the precision and accuracy data obtained from two experienced independent analysts.

Table 7 shows the results of the samples prepared by the Quality Manager and presented to an experienced analyst for analysis. In the absence of true certified reference material and incurred sample material, the results indicate that the method can be used by an experienced analyst to detect and confirm all the nitroimidazoles and the metabolites in any turkey sample. There were no false negative or false positive results for all the nitroimidazoles. However, the quantitative results for RNZ and CNZ, the two compounds that were also susceptible to significant matrix effects by either sulphonamides or thyreostats, did not meet the accuracy requirements for quantitative analysis; of the four samples containing RNZ prepared for analysis, not one of them could be quantified to the degree of accuracy required. Of the six CNZ containing samples prepared for analysis, only one (16 %) was accurately quantified. On the basis of these findings, it was concluded that both RNZ and CNZ did not meet the minimum accuracy requirements for quantitative analysis. Notwithstanding, the method was still considered suitable for making qualitative decisions about their presence and/or absence in turkey muscle tissue samples.

Conclusion

The method developed and validated for the determination of nitroimidazole residues and their 3 hydroxy metabolites in meat was simple and permitted 24 samples to be processed easily in 3 h. The resulting sample extracts were clean, allowing the confirmation of residues as low as 0.02 ng/g. The method has also been shown to be applicable to other matrices, including chicken muscle, porcine muscle, kidney and liver with no method modifications. Work is in progress to transfer and implement the method for use in the Canadian National Chemical Residue Monitoring Program (NCRMP).

Conflicts of interest

The authors have no conflicts of interest to declare.

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