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Determination of Benzimidazole Residues in Animal Tissue by Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry

ASMS 2014 TP 281

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Introduction

Benzimidazoles are broad-spectrum, high efficiency, low toxicity anthelmintic. Because some benzimidazoles and their metabolites showed teratogenic and mutagenic effects in animal and target animal safety evaluation experiment, many countries have already put benzimidazoles and metabolites as the monitoring object.

This poster employed a liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method to determinate 16 benzimidazole residues in animal tissue. The method is simple, rapid and high sensitivity, which meets the requirements for the analysis of veterinary drug residue in animal tissue.

Method

Sample Preparation

(1) Animal tissue samples were extracted with ethyl acetate-50% potassium hydroxide-1% BHT
(2) The samples were treated with n-hexane for defatting and further cleaned-up on MCX solid phase (SPE) cartridge.
(3) The separation of benzimidazoles and their metabolites was performed on LC-MS/MS instrument.

LC/MS/MS Analysis

The analysis was performed on a Shimadzu Nexera UHPLC instrument (Kyoto, Japan) equipped with LC-30AD pumps, a CTO-30A column oven, a DGU-30A5 degasser, and an SIL-30AC autosampler. The separation was carried out on a Shim-pack XR-ODS III (2.0 mmI.D. x 50 mmL., 1.6 µm, Shimadzu) with the column temperature at 30 °C. A triple quadrupole mass spectrometer (Shimadzu LCMS-8040, Kyoto, Japan) was connected to the UHPLC instrument via an ESI interface.

Analytical Conditions

UHPLC (Nexera system)

Column	: Shim-pack XR-ODS III (2.0 mmI.D. x 50 mmL., 1.6 µm)
Mobile phase A	: water with 0.1% formic acid
Mobile phase B	: acetonitrile
Gradient program	: as in Table 1
Flow rate	: 0.4 mL/min
Column temperature	: 30 °C
Injection volume	: 20 µL

Table 1 Time program

Time (min)	Module	Command	Value
0.01	Pumps	Pump B Conc.	5
3.50	Pumps	Pump B Conc.	80
4.00	Pumps	Pump B Conc.	80
4.01	Pumps	Pump B Conc.	5
6.00	Controller	Stop	

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MS/MS (LCMS-8040 triple quadrupole mass spectrometer)

Ionization	: ESI
Polarity	: Positive
Ionization voltage	: +4.5 kV
Nebulizing gas flow	: 3.0 L/min
Heating gas pressure	: 15.0 L/min
DL temperature	: 200 °C
Heat block temperature	: 350 °C
Mode	: MRM

Table 2 MRM parameters of 16 benzimidazoles (*: for quantitation)

Compound	Precursor *m/z*	Product *m/z*	Dwell Time (ms)	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
Fenbendazole	300.10	268.05*	50	-15.0	-21.0	-18.0
		159.05	50	-15.0	-36.0	-30.0
Albendazole sulfoxide	282.00	240.10*	10	-14.0	-12.0	-17.0
		208.05	10	-14.0	-23.0	-22.0
Thiabendazole	202.00	175.10*	10	-30.0	-24.0	-18.0
		131.15	10	-30.0	-31.0	-25.0
Thiabendazole-5-hydroxy	218.00	191.05*	50	-30.0	-23.0	-13.0
		147.10	50	-30.0	-32.0	-27.0
Oxfendazole	316.20	159.15*	20	-11.0	-34.0	-30.0
		191.15	20	-11.0	-22.0	-20.0
Albendazole	266.30	234.10*	8	-30.0	-19.0	-25.0
		191.10	8	-30.0	-33.0	-20.0
Albendazole -2-aminosulfone	240.30	133.20*	50	-15.0	-27.0	-24.0
		198.10	50	-15.0	-18.0	-21.0
Albendazole sulfone	298.30	159.10*	20	-13.0	-37.0	-30.0
		224.05	20	-13.0	-27.0	-23.0
Mebendazole	296.30	264.15*	10	-13.0	-21.0	-27.0
		105.25	10	-13.0	-35.0	-19.0
Mebendazole-amine	238.30	105.20*	10	-15.0	-26.0	-20.0
		133.20	10	-15.0	-36.0	-25.0
5-Hydroxymebendazole	298.30	266.10*	10	-30.0	-22.0	-18.0
		160.15	10	-30.0	-35.0	-30.0
Flubendazole	314.30	282.15*	10	-14.0	-22.0	-19.0
		123.15	10	-14.0	-35.0	-24.0
2-Aminoflubendazole	256.30	123.20*	10	-16.0	-26.0	-22.0
		95.20	10	-16.0	-41.0	-18.0
Cambendazole	303.20	217.15*	5	-30.0	-28.0	-23.0
		261.10	5	-30.0	-17.0	-28.0
Oxibendazole	250.30	218.15*	5	-30.0	-17.0	-23.0
		176.15	5	-30.0	-27.0	-18.0
Oxfendazole	332.20	300.10*	10	-15.0	-22.0	-21.0
		159.05	10	-15.0	-39.0	-30.0

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Results and Discussion

A liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) method has been developed to identify and quantify trace levels of 16 benzimidazoles residue (fenbendazole, albendazole sulfoxide, thiabendazole, thiabendazole- 5-hydroxy, oxfendazole, albendazole, albendazole-2-aminosulfone, albendazole sulfone, mebendazole, mebendazole-amine, 5-hydroxymebendazole, flubendazole, 2-aminoflubendazole, cambendazole, oxibendazole, oxfendazole) in animal tissue. The MRM chromatograms of 16 drugs mixture are presented in Fig.1. The correlation coefficients for 16 drugs (0.5 – 50 ng/mL) were found to 0.9993~0.9999. MRM chromatograms of pork samples and pork samples spiked with standards are shown in Fig.2. By analyzing 16 drugs at three levels including 0.5 ng/mL, 5 ng/mL, 50 ng/mL, excellent repeatability was demonstrated with the %RSD being better than 5% for all the compound within six injections as shown in Table 3. Results of recovery test were good as shown in Table 4.

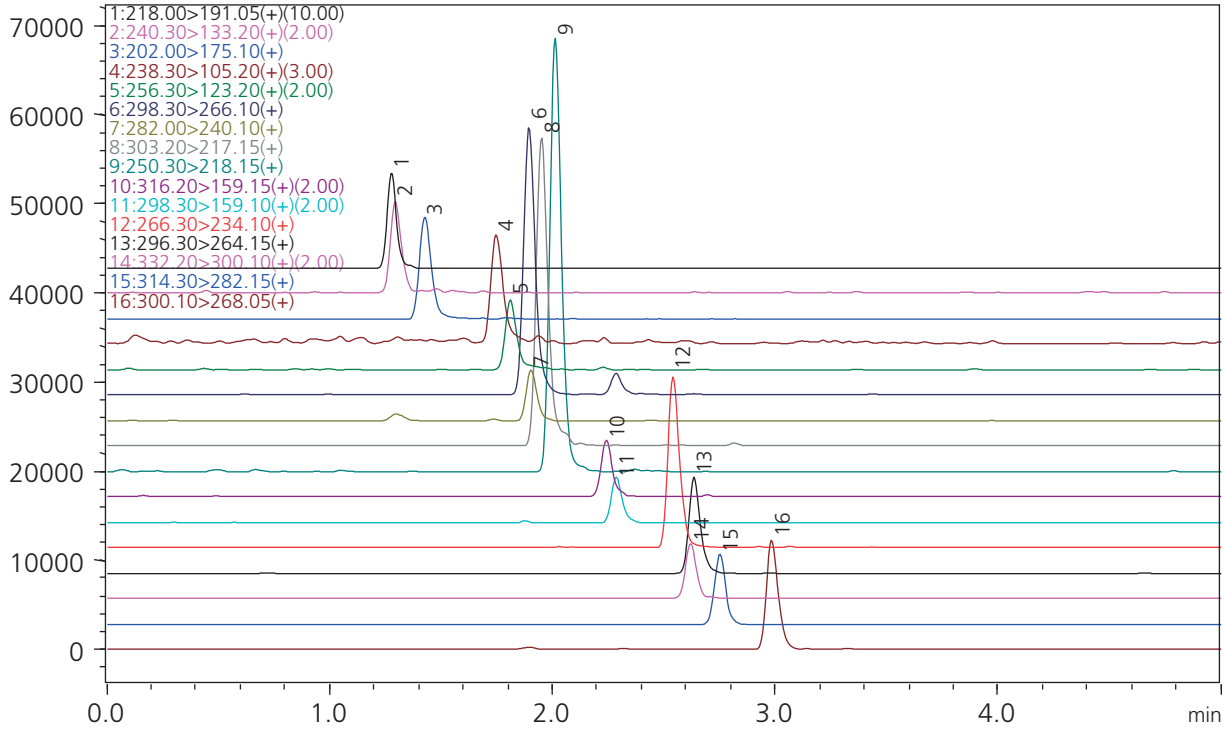

Figure 1 MRM chromatograms of standard 16 drugs (1 ng/mL)
(1: Thiabendazole-5-hydroxy; 2: Albendazole -2-Aminosulfone; 3: Thiabendazole;
4: Mebendazole-amine; 5: 2-Aminoflubendazole;6: 5-Hydroxymebendazole;
7: Albendazole Sulfoxide; 8: Cambendazole; 9: Oxibendazole; 10: Oxfendazole;
11: Albendazole sulfone; 12: Albendazole; 13: Mebendazole; 14: Oxfendazole;
15: Flubendazole; 16: Fenbendazole)

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Table 3 Repeatability of 16 drugs in pork sample (n=6)

Compound	%RSD (0.5 ng/mL)		%RSD (5.0 ng/mL)		%RSD (50 ng/mL)	
	R.T.	Area	R.T.	Area	R.T.	Area
Fenbendazole	0.059	3.01	0.064	1.48	0.082	0.34
Albendazole Sulfoxide	0.202	4.26	0.084	2.86	0.153	0.92
Thiabendazole	0.272	4.52	0.180	2.85	0.132	2.58
Thiabendazole-5-hydroxy	0.526	4.44	0.249	3.91	0.158	1.41
Oxfendazole	0.121	2.71	0.089	2.91	0.105	0.97
Albendazole	0.073	2.07	0.090	1.29	0.099	0.92
Albendazole -2-Aminosulfone	0.392	4.36	0.162	2.08	0.177	1.72
Albendazole sulfone	0.103	3.95	0.126	0.63	0.113	0.64
Mebendazole	0.093	4.95	0.095	1.69	0.094	0.74
Mebendazole-amine	0.363	3.95	0.149	2.72	0.243	0.94
5-Hydroxymebendazole	0.091	2.31	0.099	0.79	0.140	1.17
Flubendazole	0.107	4.22	0.058	1.52	0.091	1.00
2-Aminoflubendazole	0.339	4.30	0.177	2.53	0.166	1.43
Cambendazole	0.150	4.90	0.123	3.38	0.121	1.87
Oxibendazole	0.091	3.46	0.108	1.31	0.125	1.20
Oxfendazole	0.170	3.23	0.044	3.09	0.084	0.80

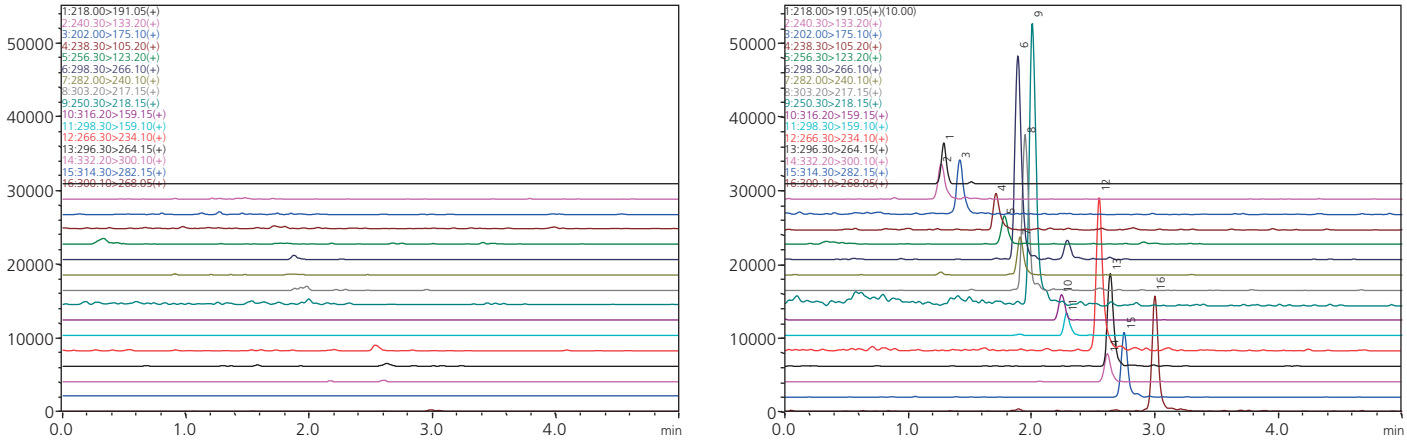

Figure 2 MRM chromatograms of pork sample (left) and spiked pork sample (right)
(1: Thiabendazole-5-hydroxy; 2: Albendazole -2-Aminosulfone; 3: Thiabendazole;
4: Mebendazole-amine; 5: 2-Aminoflubendazole;6: 5-Hydroxymebendazole;
7: Albendazole Sulfoxide; 8: Cambendazole; 9: Oxibendazole; 10: Oxfendazole;
11: Albendazole sulfone; 12: Albendazole; 13: Mebendazole; 14: Oxfendazole;
15: Flubendazole; 16: Fenbendazole)

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Table 4 Recovery of 16 drugs in pork sample

Compound	Sample Conc. (µg/kg)	Spike Conc. (µg/kg)	Measured Conc. (µg/kg)	Recovery (%)
Fenbendazole	N.D.	10.0	9.5	94.5
Albendazole Sulfoxide	N.D.	10.0	8.1	80.9
Thiabendazole	N.D.	10.0	9.8	98.2
Thiabendazole-5-hydroxy	N.D.	10.0	10.0	99.8
Oxfendazole	N.D.	10.0	11.4	113.8
Albendazole	N.D.	10.0	9.6	96.3
Albendazole -2-Aminosulfone	N.D.	10.0	9.6	96.1
Albendazole sulfone	N.D.	10.0	11.8	118.5
Mebendazole	N.D.	10.0	11.3	112.8
Mebendazole-amine	N.D.	10.0	11.8	118.3
5-Hydroxymebendazole	N.D.	10.0	9.8	97.8
Flubendazole	N.D.	10.0	10.4	103.6
2-Aminoflubendazole	N.D.	10.0	9.3	92.6
Cambendazole	N.D.	10.0	10.8	107.8
Oxibendazole	N.D.	10.0	9.6	96.1
Oxfendazole	N.D.	10.0	9.1	90.7

Conclusion

The sensitive and reliable LC/MS/MS technique was successfully applied for determination of 16 benzimidazoles residue. The calibration curves of 16 benzimidazoles ranging from 0.5 to 50 ng/mL were established and the correlation coefficients were 0.9993~0.9999. The LODs of the 16 benzimidazoles were 1 -2.2 µg/kg. The recoveries were in the range of 80.9%~118.5% for pork samples, with relative standard deviations less than 5%.

First Edition: June, 2014

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