Development of an Analytical Method for Determination of Antiparasitics Residues in Milk Using QuEChERS and Analysis by LC-MS/MS

Application Note

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Abstract

This application note describes a fast analytical method for determination of antiparasitics residues in milk using QuEChERS and analysis by liquid chromatography tandem mass spectrometry (LC-MS/MS). The method was developed and validated for simultaneous assessment of ivermectin, abamectin, doramectin, eprinomectin, moxidectin, and diflubenzuron residues in milk. The limits of quantification (LOQs) were lower than 2.5 µg/L for all compounds. The recovery ranged from 75.0 to 122.0%, and RSD were lower than 8.0%.

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Introduction

There is an increasing demand for residue-free food. The presence of veterinary drugs in animal origin foods has been widely studied, and the establishment of rigid regulations regarding maximum residue levels has been a constant. Monitoring the presence of these compounds has an important role in quality control of food, and demands a method capable of detecting these compounds in smaller levels. This application note presents the development and validation of a methodology for the analysis of six antiparasitics drugs (ivermectin, abamectin, doramectin, eprinomectin, moxidectin, and diflubenzuron) used in dairy cattle.

Residues of veterinary drugs in food are monitored by regulatory agencies using established maximum residue limits (MRLs). For the CODEX ALIMETARIUS, no MRL was set for moxidectin in milk, while the MRLs values for abamectin, ivermectin, doramectin, eprinomectin, and diflubenzuron were established as 5.0 µg/L, 10 µg/L, 15 µg/L, 20 µg/L, and 20 µg/L, respectively [1]. In Brazil, the veterinary drugs are monitored by the National Residue Control Plan (PNCR), adopting the MRL values suggested by CODEX, Food and Drug Administration (FDA), and European Union (EU). In the Brazilian PNCR, the MRL values for abamectin, ivermectin, and moxidectin in milk were established as 10.0 µg/L. For doramectin and eprinomectin, the MRL was set as 15 and 20 µg/L, respectively [2]. There is no MRL value set for diflubenzuron.

Experimental

LC conditions

Instrument	Agilent 1290 Infinity LC system
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Column temperature	40 °C
Injection volume	20 µL
Mobile phase	A) Ammonium formate (20 mM) B) Acetonitrile

Gradient	Time (min)	A (%)	B (%)
	0	30	70
	4	10	90
	6	10	90
	6.01	30	70

Flow rate	0.3 mL/min

MS conditions

Instrument	Agilent 6460 MS
Ion mode	AJS-ESI, positive ionization
Capillary voltage	5,000 V
Drying gas (N_2)	5 L/min
Drying gas temperature	250 °C
Nebulizer	30 psi
Sheath gas heater	200 °C
Sheath gas flow	7 L/min

Table 1 shows the monitored ions for each compound. The most intense transition was used as a quantifying ion, and the second most intense was used as a qualifying ion for the confirmation of the analysis.

Table 1. Retention Time (RT) and MRM Conditions of Selected Compounds

Analyte	RT (min)	Precursor (*m/z*)	Transition (*m/z*)	CE (eV)
Diflubenzuron	0.87	311	157	8
			112	64
Eprinomectin	1.94	914.5	185.6	13
			153.6	41
Abamectin	2.60	890.5	566.6	9
			304.5	21
Doramectin	3.16	916.5	592	9
			330.4	21
Moxidectin	3.54	642.4	528	0
			497	5
Ivermectin	4.03	892.5	568	0
			306.5	21

Sample preparation

Extraction of the antiparasitic drugs from milk was performed using the QuEChERS method. A 10-mL aliquot of milk sample was placed into a 50-mL centrifuge tube followed by extraction using 10.0 mL of acetonitrile. The partition step was performed by adding 4.0 g of anhydrous magnesium sulphate ($MgSO_4$) and 1.0 g of sodium chloride (NaCl) with consecutive shaking for 1 minute, and centrifugation for 5 minutes at 4,000 rpm. The cleanup step was taken with 2 mL of the supernatant into a 15-mL PP tube containing 100 mg of PSA sorbent and 300 mg of $MgSO_4$, shaken in vortex for 1 minute, and centrifuged for 5 minutes at 4,000 rpm. After that, the extract was filtered and injected.

Results and Discussion

Figure 1 shows the MRM chromatograms obtained for the mixture of ivermectin, abamectin, doramectin, and eprinomectin at 30 µg/L, moxidectin at 150 µg/L, and diflubenzuron at 250 µg/L in milk extract.

The linearity of the analytical curve was studied using matrix-matched antiparasitics standard solutions in seven concentrations ranging from 1.0 to 30.0 µg/L for ivermectin, abamectin, doramectin, and eprinomectin, from 5.0 to 150.0 µg/L for moxidectin, and from 10.0 to 250.0 µg/L for diflubenzuron. For all compounds, the determination coefficient (R^2) calculated by linear regression presented values greater than 0.98. Figure 2 shows an example of the response for antiparasitics drugs in milk matrix.

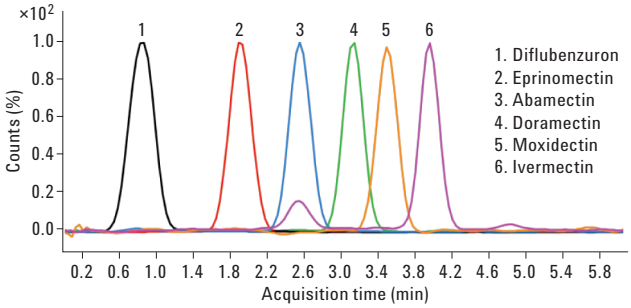

Figure 1. Normalized LC-MS/MS chromatogram of (1) diflubenzuron, (2) eprinomectin, (3) abamectin, (4) doramectin, (5) moxidectin, and (6) ivermectin standard solutions spiked in milk sample.

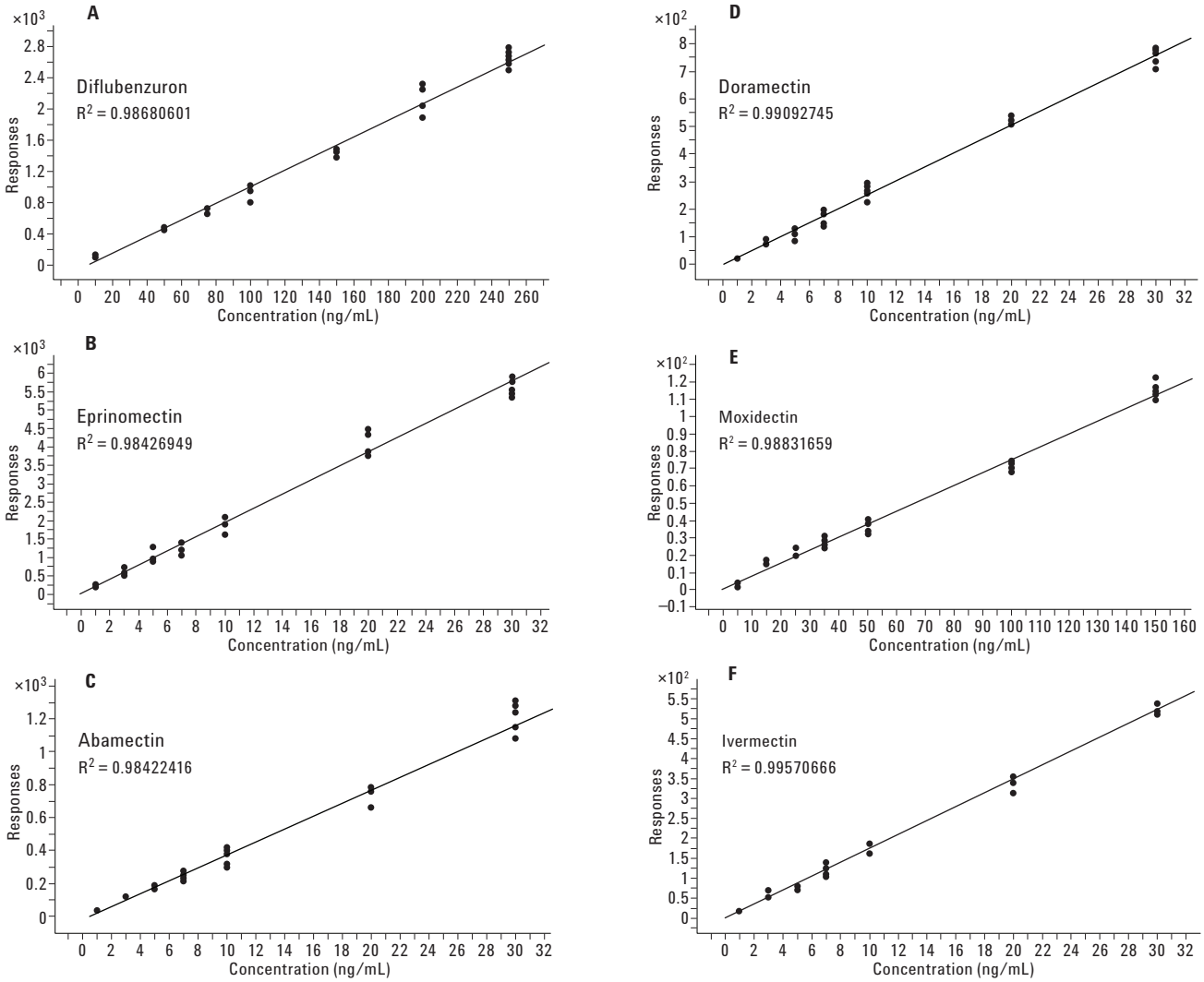

Figure 2. Calibration curves of (A) diflubenzuron, (B) eprinomectin, (C) abamectin, (D) doramectin, (E) moxidectin, and (F) ivermectin in milk sample.

Precision and accuracy expressed in terms of recovery from milk were studied by analyzing spiked samples at three different levels of concentration in five replicate measurements. These results as well the limits of detection (LOD) and LOQ obtained are presented in Table 2.

Table 2. Percentage of Recoveries for Three Fortification Levels in Milk, RSD (%), LOD, and LOQ of Antiparasitics Drugs in Milk

Analyte	Level (µg/L)	Recovery (%)	RSD (%)	LOD (µg/L)	LOQ (µg/L)
Diflubenzuron	10.0	104	0.3	0.8	2.5
	15.0	101	1		
	20.0	87	1		
Eprinomectin	1.0	122	7	0.08	0.3
	5.0	86	4		
	10.0	75	4		
Abamectin	1.0	116	3	0.2	0.7
	5.0	98	4		
	10.0	95	2		
Doramectin	1.0	94	5	0.1	0.4
	5.0	99	2		
	10.0	102	8		
Moxidectin	5.0	100	6	0.6	1.9
	25.0	97	3		
	50.0	94	7		
Ivermectin	1.0	102	2	0.1	0.4
	5.0	102	6		
	10.0	98	2		

Conclusion

The proposed method takes less than 5 minutes for analysis, and has been successfully applied to the determination of antiparasitics drugs in samples of milk. The sensitivity and specificity of the method are suitable to meet the residue limits established in most countries. The proposed methodology is simple, quick, and presents linear calibration curves and excellent precision data for replicate injections.

References

1. http://www.codexalimentarius.net.
2. BRASIL, Ministério da Agricultura, Pecuária e Abastecimento, Secretaria de Defesa Agropecuária, Instrução Normativa No. 11/2012.

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