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Applikationsbericht

QuEChERS Sample Preparation for LC-MS/MS Determination of Avermectins in Meat and Milk

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Abstract

In this application note, milk and ground beef are prepared and analyzed for avermectins at the ppb level using QuEChERS methodology and LC-MS/MS.

Benefits

- Simple, high throughput preparation of meat and milk samples using QuEChERS methodology
- Sensitive LC-MS/MS analysis of avermectins in complex sample matrices using fast and easy DisQuE sample preparation
- Achieve part-per-billion (ppb) level detection of avermectins to meet country-specific maximum residue limits (MRL)
- Sample preparation using DisQuE products conforms to CEN method 15662

Introduction

Avermectins are sixteen-membered ring macrolide drugs that are used as veterinary antihelmintics. The lowest allowable limit for these compounds in food products is set based on worldwide safety evaluations. Regulatory MRLs for avermectins can vary worldwide but are generally in the ppb concentration range. The sensitive analysis of avermectins in food products such as milk and meat can be challenging due to their complex sample matrices.

QuEChERS is a simple and straightforward sample preparation technique that involves a salting-out liquid extraction followed by optional dispersive solid-phase extraction (d-SPE). Sample preparation using QuEChERS allows for fast throughput and high sensitivity analysis of food products. Although QuEChERS is commonly used for multi-residue pesticide analysis in fruits and vegetables, it is also applicable in the analysis of veterinary drugs in livestock products. In this application note, milk and ground beef are prepared and analyzed for avermectins at the ppb level using QuEChERS methodology and LC-MS/MS.

Experimental

UPLC conditions

System:	ACQUITY UPLC System
Column:	XSelect CSH C_{18} *XP*, 130 Å, 2.5 µm, 2.1 x 100 mm (p/n 186006103)
Injection volume:	5 µL
Temperature:	50 °C
Mobile phase:	A: 5 mM ammonium acetate in water B: 5 mM ammonium acetate in methanol
Flow rate:	0.40 mL/min
Gradient:	70% B initial, linear gradient to 97% B in 5 minutes, hold until 8 minutes, back to 70% B at 8.1 minutes. Hold and re-equilibrate until 10 minutes
Sample vials:	Maximum Recovery Vial (p/n 600000670CV)

MS conditions

System:	Xevo TQ-S Mass Spectrometer
Ionization mode:	Electrospray positive (ESI+)

The MRM transitions, cone voltages, and collision cell energies optimized for avermectins in this study are presented below.

Compound Name	MRM Transition (*m/z*)	Cone (V)	Collision (eV)
Abamectin	890.5 > 305.5	35	17
Ivermectin	892.5 > 307.5	30	20
Doramectin	916.5 > 331.2	10	20
Eprinomectin	914.5 > 186.2	20	17
Moxidectin	640.3 > 528.3	10	5

Table 1. MRM transitions.

Sample preparation

Initial Extraction (QuEChERS)

Place 10 mL whole milk (pasteurized) into a 50 mL centrifuge tube, or for meat, place 8 g ground beef (80% lean) and 2 mL water into a 50 mL centrifuge tube. Add 10 mL acetonitrile and shake the tube vigorously for 1 minute. Add the contents of DisQuE pouch salts for European Committee for Standardization (CEN) QuEChERS (part number: 186006813) and shake vigorously for 1 minute. Centrifuge for 15 minutes at 4000 rpm and take a 1 mL aliquot of the supernatant (top layer) for d-SPE cleanup.

d-SPE Cleanup

Transfer the 1 mL aliquot of supernatant to a 2 mL d-SPE cleanup tube that contains 150 mg magnesium sulfate and 50 mg C_{18} sorbent and shake vigorously for 1 minute. Centrifuge for 5 minutes at 12000 rpm and take a 0.5 mL aliquot a a sample for LC-MS/MS analysis.

Results and Discussion

Recovery was measured for each steroid hormone at both low and high concentration levels (Table 2). Recovery was calculated by comparing the MRM peak area for samples spiked prior to QuEChERS extraction (pre-spiked samples) with the MRM peak area for samples spiked after QuEChERS extraction (post-spiked samples). Table 3 shows the calculated matrix effect for each compound. Matrix effects were calculated by comparing the MRM peak area of post-spiked samples with the MRM peak area for equivalent standards prepared in acetonitrile. Figures 1 and 2 show LC-MS/MS chromatograms obtained from the analysis of meat and milk samples, respectively, spiked with the low concentration level of avermectins.

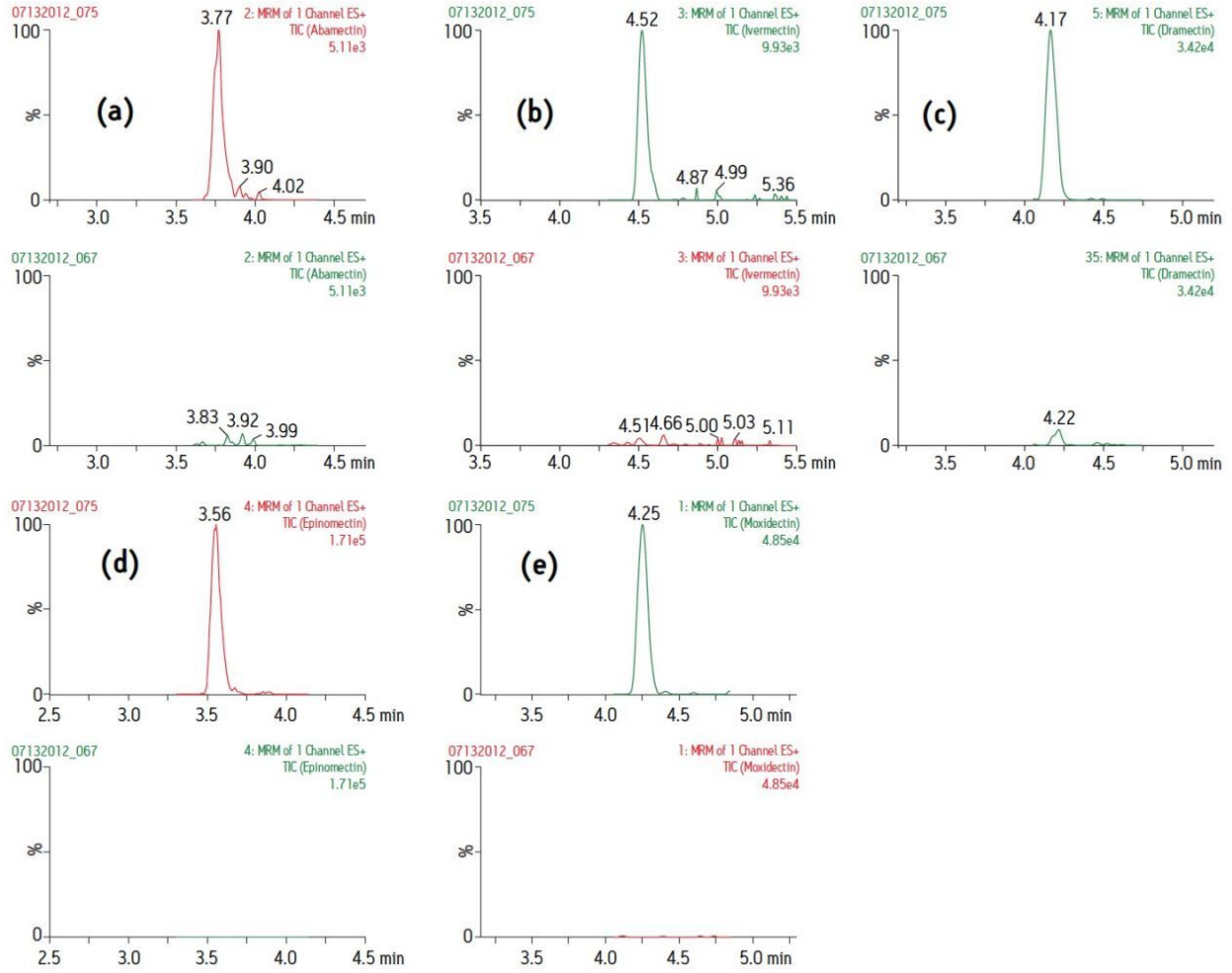

Figure 1. LC-MS/MS chromatograms of avermectins obtained from ground beef samples; the top trace is the low level spiked sample, the bottom trace is a ground beef blank. (a) abamectin (b) ivermectin (c) doramectin (d) eprinomectin (e) moxidectin.

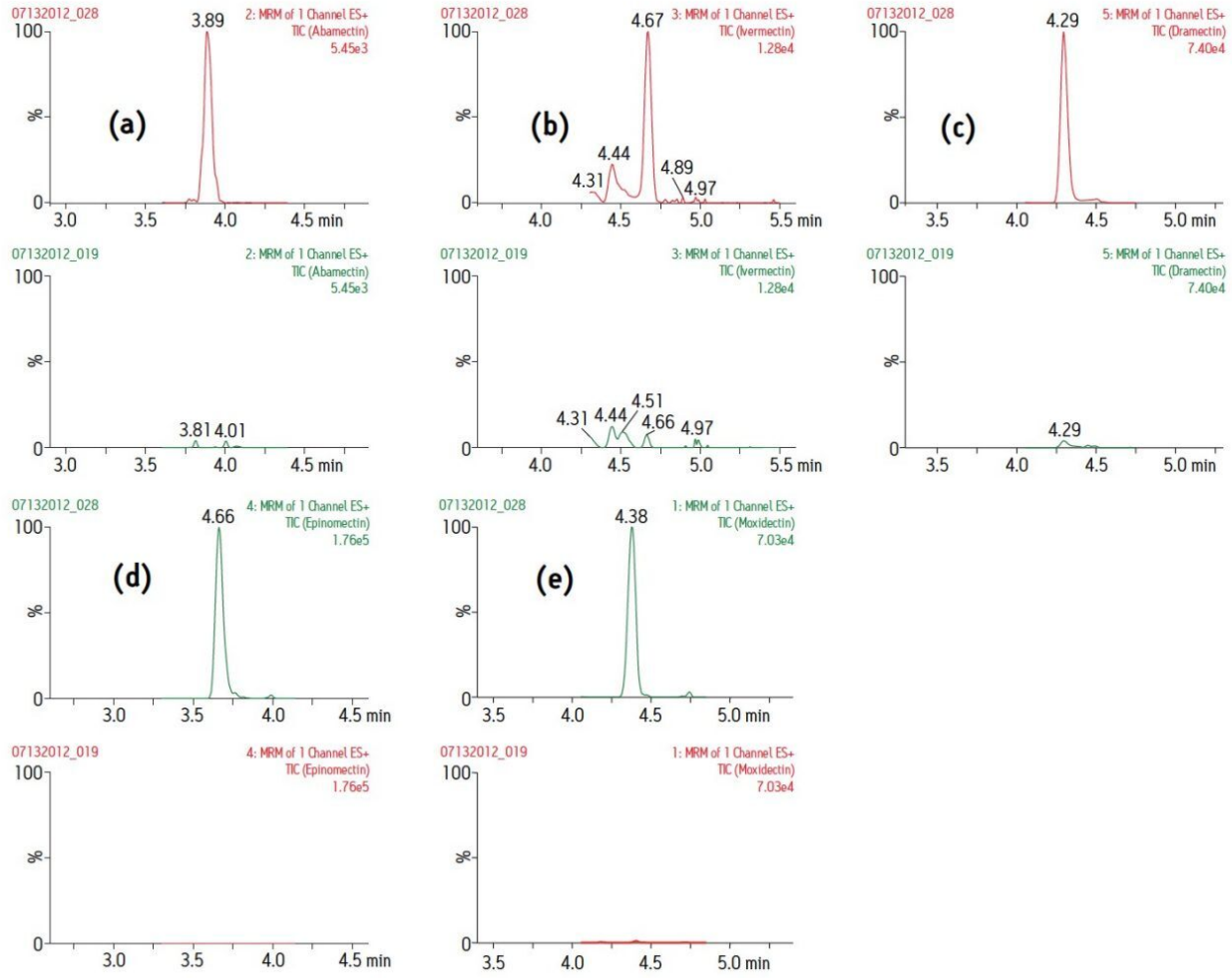

Figure 2. LC-MS/MS chromatograms of avermectins obtained from whole milk samples; the top trace is the low level spiked sample, the bottom trace is a whole milk blank. (a) abamectin (b) ivermectin (c) doramectin (d) eprinomectin (e) moxidectin.

	Concentration Range (ppb)		Average % Recovery (%RSD) n=5			
			Ground Beef		Whole Milk	
Conc. Level	Low Level	High Level	Low Level	High Level	Low Level	High Level
Abamectin	1	10	94(3.6)	88(3.6)	86(14.0)	89(3.7)
Ivermectin	1	10	98(17.7)	85(3.1)	84(5.3)	83(14.8)
Doramectin	10	100	89(4.8)	85(4.2)	101(11.7)	90(5.0)
Epinomectin	10	100	99(2.9)	91(1.5)	94(3.9)	93(3.0)
Moxidectin	10	100	90(4.2)	87(1.8)	100(2.4)	90(5.6)

Table 2. Recoveries of avermectins from ground beef and whole milk samples.

Conc. Level	Concentration Range (ppb)		Matrix Effect (%)* n=5			
			Ground Beef		Whole Milk	
	Low Level	High Level	Low Level	High Level	Low Level	High Level
Abamectin	1	10	105	96	109	93
Ivermectin	1	10	73	95	83	95
Doramectin	10	100	96	92	109	105
Epinomectin	10	100	127	131	92	100
Moxidectin	10	100	101	94	71	85

Table 3. Matrix effects of avermectins from ground beef and whole milk samples.

** Matrix effect (%) = peak area (post-spiked sample)/peak area (standard) x 100*

A value > 100% indicates ionization enhancement

A value < 100% indicates ionization suppression

For the analysis of abamectin, ivermectin, doramectin, eprinomectin, and moxidectin in challenging matrices like whole milk and ground beef, good recoveries were obtained using DisQuE sample preparation sorbent pouches. QuEChERS extraction is a simple and straightforward method for sample preparation and has been shown to be effective for the extraction of avermectins from milk and meat. The incorporation of a d-SPE clean-up step prior to LC-MS/MS analysis aids in the reliable quantitation of avermectins at low ppb concentrations in complex matrices.

Conclusion

- The DisQuE pouch (QuEChERS) protocol is an easy and effective method for the extraction of avermectins from livestock products.
- DisQuE sample preparation provides excellent sample cleanup prior to LC-MS/MS analysis, with high analyte recoveries and minimal matrix effects.
- DisQuE extraction combined with LC-MS/MS analysis using e*X*tended *P*erformance [*XP*] 2.5 µm columns provides low ppb quantitation of avermectins in complex matrices, enabling reliable testing.

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