Determination of Anthelmintic Drug Residues in Milk Using Ultra High Performance Liquid Chromatography Tandem Mass Spectrometry*

UCT Part Numbers

ECMSSC50CT-MP
4000 mg anhydrous $MgSO_4$
1000 mg NaCl

ECMSC1850CT
1500 mg anhydrous $MgSO_4$
and 500mg C18

Introduction:

A modified QuEChERS-based method is used with an additional concentration step to detect 38 anthelmintic residues (nematicides, flukicides, endectocides) in milk at ≤1µg/kg using UHPLC-MS/MS detection. The drugs covered by this method include benzimidazoles, avermectins and flukicides.

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Procedure:

1. Sample Preparation

a) Weigh 10.0 grams milk into a 50 mL centrifuge tube
b) Add IS and allow to sit for 15 minutes
c) Add 10 mL acetonitrile (MeCN) and the contents of **ECMSSC50CT-MP** pouch
d) Shake vigorously, then centrifuge for 12 minutes @ ≥ 3,500 rcf

2. Dispersive Sample Cleanup

a) Add the supernatant to **ECMSC1850CT**
b) Vortex sample for 30 seconds
c) Centrifuge for 10 minutes @ ≥ 3000 rcf
d) Transfer 5 mL of supernatant to an evaporation tube
e) Add 0.25 mL DMSO (keeper solvent) and vortex briefly
f) Evaporate the MeCN @ 50° C using nitrogen evaporation to 0.25 mL
g) Filter extract using 0.2 µm PTFE syringe filter
h) Sample is ready for UHPLC-MS/MS analysis

Analysis UHPLC-MS/MS:

UHPLC-MS/MS
UPLC: Waters Acquity UPLC system (Milford MA; USA) or equivalent
Analytical Column: HSS T3 C18 (100 × 2.1 mm, particle size 1.8 µm) (or equivalent) with appropriate guard column
Column Temperature: 60 °C
Pump Flow rate: 0.6 mL/min
Autosampler temperature: 10 °C
Injection volume: 5 µL
Mass spectrometer: Waters Quattro Premier XE triple quadrupole
Ionization mode: Electrospray ionization (ESI) interface using fast polarity switching
Software: System controlled by MassLynx™ software and data was processed using TargetLynx™ Software (Waters)

Gradient Profile		
Time (min)	**Mobile Phase A** (0.01 % formic acid in water:MeCN (90:10, v/v)	**Mobile Phase B** (5mM ammonium formate in MeOH:MeCN (75:25 v/v)
0 - 0.5	100 %	
5	50 %	
7	10 %	
8.5	10 %	
8.51	0 %	
9.5	0 %	
9.51	100 %	
13	100 %	

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Notes:
Ammonium formate is used in the organic mobile phase because abamectin, doramectin and ivermectin form sodium adducts ([M+23]+) when acids are used. In this case, the ammonium adducts ([M+18]+) should be monitored for these three compounds and not the protonated precursor ions.

MS amenable acids can be used for the aqueous mobile phase, which should be at a low pH (≤4) to get the best results. It is essential to use ammonium buffer in the organic mobile phase as the avermectins elute at 100% organic content. The aqueous mobile phase may also include ammonium buffer, although it is not an essential requirement. Additionally, ammonium formate is more soluble in organic solvent than ammonium acetate.

Albendazole-sulfone and hydroxy-mebendazole are prone to isobaric interference as they have similar precursor and product ions that can't be distinguished using triple quadrupole instruments. It is therefore necessary to chromatographically separate these two compounds.

Standards, Internal Standards, Stock Solutions & Suppliers:

Sigma-Aldrich

Analyte	Abbreviation	Analyte	Abbreviation
Abamectin	ABA	Ivermectin	IVER
Albendazole	ABZ	Levamisole	LEVA
Bithionol	BITH	Morantel	MOR
Clorsulon	CLOR	Niclosamide	NICL
Closantel	CLOS	Nitroxynil	NITR
Coumaphos	COUM	Oxfendazole	OFZ
Doramectin	DORA	Oxyclozanide	OXY
Emamectin	EMA	Rafoxanide	RAF
Fenbendazole	FBZ	Thiabendazole	TBZ
Haloxon	HAL		

Witega Laboratories Berlin-Aldershof GmbH (Berlin, Germany)

Analyte	Abbreviation
Albendazole-2-amino-sulfone	ABZ-NH_2-SO_2
Albendazole sulfone	ABZ-SO_2
Albendazole-sulfoxide	ABZ-SO
Amino-oxibendazole	OXI-NH_2
5-hydroxy-thiabendazole	5-OH-TBZ
Fenbendazole-sulfone	FBZ-SO_2
Triclabendazole	TCB
Triclabendazole-sulfone	TCB-SO_2
Triclabendazole sulfoxide	TCB-SO

Deuterated forms of these standards are available from Witega & QUCHEM (Belfast, UK)

Janssen Animal Health (Beerse, Belgium)

Analyte	Abbreviation
Amino-flubendazole	FLU-NH_2
Amino-mebendazole	MBZ-NH_2
Hydroxy-flubendazole	FLU-OH
Hydroxy-mebendazole	MBZ-OH
Flubendazole	FLU
Mebendazole	MBZ

Greyhound Chromatography and Allied Chemicals, (Merseyside, UK)

Analyte	Abbreviation
Coumaphos-oxon	COUM-O

QMX Laboratories (Essex, UK)

Analyte	Abbreviation
Cambendazole	CAM
Oxibendazole	OXI

Merial Animal Health (Lyon, France)

Analyte	Abbreviation
Eprinomectin	EPR

Fort Dodge Animal Health (Princeton, NJ, USA)

Analyte	Abbreviation
Moxidectin	MOXI

Non-Isotopically Labeled Internal Standards Used

Internal Standard	Abbreviation & Source
Selamectin	SELA (Pfizer Animal Health)
Salicylanide	SALI (Sigma-Aldrich)
4-nitro-3-(trifluoromethyl)phenol	TFM (Sigma-Aldrich)
Ioxynil	IOX (Sigma-Aldrich)

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Primary Stock Standard Solutions:

- 4,000 µg/mL from the certified standard materials-- ABZ, ABZ-SO, ABZ-SO2, ABZ-NH_2-SO_2, FBZ, OFZ, FBZ-SO2, EPR, CLOS, OXY, NITR, CLOR, BITH and MOR
- The remaining standards are prepared at concentrations of 2,000 µg/mL
- All internal standards are prepared at concentration of 1,000 µg/mL
- Avermectins were prepared in MeCN
- Flukicides, CAM, LEVA and TCB metabolites are prepared in MeOH
- Benzimidazoles are prepared in DMSO

Intermediate working standard mix solutions:

- 100 µg/mL for OXY, CLOR, BITH and MOR
- 50 µg/mL in MeOH for the remaining analytes

Prepare working IS as follows:

- 20 µg/mL for SELA and TCB-NH_2, 4 µg/mL for LEVA-D5, TBZ164 D3 and IOX
- 2 µg/mL for the remaining analytes in MeOH-D

Primary, intermediate and working standard solutions are stable for at least six months when stored at -20°C.

References:

[1] *Adapted and used with permission from Whelen, M., Kinsella, B., "Determination Of Anthelmintic Drug Residues In Milk Using Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry With Rapid Polarity Switching", doi:10.1016/j.chroma.2010.05.007, CHROMA 351049, J. of Chromatography A

[2] **Listing of instrument manufacturers and standards suppliers does not constitute endorsement by UCT. Equivalent systems may be used

DCN-313150-265

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

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