



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

UCT Products:

ECMSSC50CT-MP (4000 mg anhydrous MgSO₄, 1000 mg NaCl)

ECMSC1850CT (1500 mg anhydrous MgSO₄ and 500mg C18)

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Introduction

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

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 @ $\geq 3,500$ 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

3. Analysis UHPLC-MS/MS

- 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 of 0.6 mL/min
- Binary gradient:
 - 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)
 - Gradient profile:
 - 0 – 0.5 min, 100% A
 - 5 min, 50% A
 - 7 min, 10% A
 - 8.5 min, 10% A
 - 8.51 min, 0% A
 - 9.5 min, 0% A
 - 9.51 min, 100% A
 - 13 min, 100% A
- Injection volume 5 μL
- Waters Quattro Premier XE triple quadrupole mass spectrometer
- Electrospray ionization (ESI) interface using fast polarity switching
- System controlled by MassLynx™ software and data was processed using TargetLynx™ Software (Waters)

Note:

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 | Nitroxylnil | 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) |

Primary Stock Standard Solutions:

- 4,000 µg/mL from the certified standard materials-- ABZ, ABZ-SO, ABZ-SO₂, ABZ-NH₂-SO₂, 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-NH₂, 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

*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

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

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