



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

UCT Products:

**ECMSSC50CT-MP** (4000 mg anhydrous MgSO<sub>4</sub>, 1000 mg NaCl)

**ECMSC1850CT** (1500 mg anhydrous MgSO<sub>4</sub> and 500mg C18)

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### Introduction

A modified QuEChERS-type extraction method is used with an additional concentration step to detect 38 anthelmintic (nematicides, flukicides, endectocides) drug residues, consisting of benzimidazoles, avermectins and flukicides in milk. UHPLC-MS/MS instrumentation detects most analytes at < 1µg/kg

### 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 12 mLs acetonitrile (MeCN) and the contents of **ECMSSC50CT-MP** pouch
- d) Shake vigorously then centrifuge for 12 minutes @ 3,500 rpm

#### 2) Dispersive Sample Cleanup

- a) Add the supernatant to **ECMSC1850CT** in a 50 mL centrifuge tube
- b) Vortex sample for 30 seconds
- c) Centrifuge for 10 minutes @ 2,500 rpm (1,449 g)
- d) Transfer 6 mL of supernatant to an evaporation tube
- e) Add 0.25 mLs DMSO and vortex for 1 minute
- f) Evaporate the MeCN @ 50° C using a Turbovap® or similar apparatus to 0.25 mLs
- g) Filter extract using 0.2 µm PTFE a syringe filter
- h) Sample is ready for UHPLC analysis

### 3) Analysis UPLC-MS/MS

- Waters Acquity UPLC system (Milford MA; USA) or equivalent
- Analytical column SS HSS T3 (or equivalent) (100 × 2.1 mm, particle size 1.8 µm) with in-line filter unit and 0.2 µm stainless steel filter
- Maintain column at 60° C
- Pump flow rate of 0.6 mL/min
- Binary gradient:
  - mobile phase A 0.01% HOAc 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
- 200 electrospray ionization (ESI) interface
- System control 201 by MassLynx™ software and data was processed using TargetLynx™ Software (Waters)
- Parent ion masses are calculated from the elementary compositions

### 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         | Niclozamide   | 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-sulphone | ABZ-NH <sub>2</sub> -SO <sub>2</sub> |
| Albendazole sulphone         | ABZ-SO <sub>2</sub>                  |
| Albendazole-sulphoxide       | ABZ-SO                               |
| Amino-oxibendazole           | OXI-NH <sub>2</sub>                  |
| 5-hydroxy-thiabendazole      | 5-OH-TBZ                             |
| Fenbendazole-sulphone        | FBZ-SO <sub>2</sub>                  |
| Triclabendazole              | TCB                                  |
| Triclabendazole-sulphone     | TCB-SO <sub>2</sub>                  |
| Triclabendazole sulphoxide   | 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 <sub>2</sub> |
| Amino-mebendazole    | MBZ-NH <sub>2</sub> |
| 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         |

**Other Internal Standards**

| 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-SO<sub>2</sub>, ABZ-NH<sub>2</sub>-SO<sub>2</sub>, FBZ, OFZ, FBZ-SO<sub>2</sub>, 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 is 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<sub>2</sub>, 4 µg/mL for LEVA-D<sub>5</sub>, TBZ164 D<sub>3</sub> 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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