



Analysis of Flukicides/ Anthelmintics in Animal Tissue Using QuEChERS and LC/MS/MS

UCT Part Number:

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

ECMSC1850CT (1500 mg MgSO₄, 500 mg endcapped C18)

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Procedure

1. Extraction

- a) To 10 g of homogenized/hydrated sample in a 50 mL centrifuge tube add 10 mL acetonitrile
- b) Add internal standard (100 ng/g Cyprodinil + 2,4-D)

Note: Isotopically labeled internal standards are now commercially available.

- c) Shake for 1 minute
- d) Add contents of ECMSSC50CT-MP pouch (4 g of anhydrous magnesium sulfate and 1g sodium chloride) to the centrifuge tube
- e) Immediately shake for 1 minute
- f) Centrifuge for 5 minutes at 3450 rcf

2. Sample Clean-Up

- a) Add a 3 mL aliquot of supernatant from (step 1 f) to **ECMSC1850CT**
- b) Shake for 1 minute
- c) Centrifuge for 1 minute at 3450 rcf

3. Analysis

- a) Transfer 0.5 mL of cleaned extract into a autosampler vial
- b) Add QC spike (100 ng/mL TPP).

Note: Isotopically labeled internal standards are now commercially available.

- c) Inject onto LC-MS/MS
- d) Use ESI⁺ and/or ESI⁻ mode depending upon specific analytes of interest

Note:

Abamectin, doramectin and ivermectin form sodium adducts ($[M+23]^+$) when acids are used as mobile phase additive in MS analysis. It is advisable to use ammonium formate or ammonium acetate as mobile phase buffer and monitor the ammonium adduct ($[M+18]^+$) for these three compounds. It is essential to use ammonium buffer in the organic mobile phase as the avermectins elute at 100% organic content. In addition, ammonium formate is more soluble in organic solvent than ammonium acetate.

MS amenable acids can be used for the aqueous mobile phase, which should be at a low pH (≤ 4) to get the best results. The aqueous mobile phase may also include ammonium buffer, although it is not an essential requirement.

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.

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ESI+		ESI-
ISTD Triphenylphosphate	QC Spike Cyprodinil	ISTD 2,4D
Abamectin	Albendazole	Bithionol
Doramectin	Albendazole-sulfoxide	Clorsulon
Emamectin	Albendazole-sulfone	Closantel
Eprinomectin	Albendazole-amino-sulfone	Niclosamide
Moxidectin	Cambendazole	Nitroxynil
Ivermectin	Flubendazole	Oxyclozanide
Selamectin	Flubendazole, amino	Rafoxanide
Dichlorvos	Flubendazole, hydroxy	Triclabendazole-sulfoxide
Coumaphos	Mebendazole	
Coumaphos-oxon	Mebendazole, amino	Albendazole-amino-sulfone
Haloxon	Mebendazole, hydroxy	
Morantel	Oxibendazole	
Levamisole	Thiabendazole	
Fenbendazole	Thiabendazole, 5-hydroxy	
Fenbendazole-sulfone	Triclabendazole	
Fenbendazole-sulfoxide (oxfendazole)		

Adapted from Kinsella, Lehotay et al, "New method for the Analysis of Anthelmintics in Animal Tissue"