



QuEChERS Analysis of Miticides and Other Agrochemicals in Honey Bees, Wax or Pollen*

UCT Part Number:

ECMSSA50CT-MP (6000 mg MgSO₄ and 1500 mg sodium acetate)

CUMPSC18CT (150 mg MgSO₄, 50 mg PSA, 50 mg C18)

ECPSACB256 (dual layer cartridge 250 mg GCB, 500 mg PSA)

ECMAG00D (organic free magnesium sulfate anhydrous)

*Summarized and adapted from: Mullin CA, Frazier M, Frazier JL, Ashcraft S, Simonds R, et al. (2010) *High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health*. PLOS ONE 5(3): e9754. doi:10.1371/journal.pone.0009754

An analytical method using QuEChERS type procedures for 121 different pesticide residues is described. Extracts of wax, beebread, and adult bees or brood can also be analyzed for metabolites of primary miticides and insecticides using this method. This includes the oxon and phenolic metabolites of coumaphos, chlorferone, the sulfoxide and sulfone metabolites of aldicarb, and the toxic olefin and 5-hydroxy metabolites of imidacloprid.

Sample Collection and Preservation

- Wrap in aluminum foil and store on dry ice until placement in a -80° C freezer as soon as possible
- Beebread and brood can be removed from the combs at room temperature and then stored along with the remaining beeswax at -20° C until processed

1. Sample Preparation

- a) Weigh 3 grams beebread (or comb wax) into a 50 mL centrifuge tube
- b) Add 100 µL of a process control spiking solution
- c) Add 27 mL of extraction solution (44% DI water, 55% acetonitrile & 1% glacial acetic acid).
Brood and adults are extracted without using DI water.
- d) Add 100 µL of an internal standard
- e) For beebread, reduce particle size by using a high speed disperser for 1 minute
- f) For comb wax melt the sample at 80° C in a water bath followed by cooling to room temperature
- g) Add the contents of **ECMSSA50CT-MP** pouch to the mixture
- h) Seal tube and shake vigorously for 1 minute
- i) Centrifuge for 1 minute

2. Clean-Up for LC/MS-MS

- a) Transfer 1 mL of supernatant to **CUMPSC18CT** micro centrifuge tube
- b) Vortex for 1 minute and centrifuge
- c) Transfer supernatant to an autosampler vial for LC analysis

3. Clean-Up for GC/MS

- a) Prepare a dual layer solid-phase extraction cartridge **ECPSACB256** by adding about 80 mg of anhydrous magnesium sulfate (ECMAG00D) to the top of the cartridge
- b) Condition cartridge by adding 4.0 mL of acetone/toluene (7:3 v:v)
- c) Using a positive pressure or vacuum manifold, elute solvent to waste
- d) Add 2 mL of supernatant to the cartridge
- e) Elute cartridge using 3 x 4 mL of acetone/toluene & 7:3, (v:v) into a 15 mL graduated glass centrifuge tube
- f) Using an analytical evaporator @ 50°C, dry eluate to a final volume of 0.4 mL
- g) Sample is ready for analysis

4. Analysis—by LC or GC

LC analysis is necessary for neonicotinoids, other polar pesticides and their metabolites

For LC analysis

- a) Analysis by LC/MS-MS use a 3.5 μm 2.1 X 150 mm Agilent Zorbax SB-C18 (or equivalent)
- b) Agilent 1100 LC with a binary pump interfaced to a Thermo-Fisher TSQ Quantum Discovery triple quadrupole MS 9 (or equivalent)

For GC analysis

- a) For analysis use Agilent 6890 (or equivalent) GC equipped with a 0.25 mm, 30 m J&W DB-5MS (2 μm film) capillary column
- b) Interface to an Agilent 5975 triple quadrupole MS (or equivalent)
- c) Use GC/MS in the electron impact and negative chemical ionization modes

Pesticides Representatives Found in Wax Samples

Aldicarb sulfoxide	Flutolanil
Aldicarb sulfone	Fluvalinate
Allethrin	Heptachlor
Atrazine	Heptachlor epoxide
Azinphos methyl	Hexachlorobenzene
Azoxystrobin	Imidacloprid
Bendiocarb	Iprodione
Bifenthrin	Malathion
Boscalid	Metalaxyl
Captan	Methidathion
Carbaryl	Methoxyfenozide
Carbendazim	Metribuzin
Carbofuran	Norflurazon
Carbofuran, 3-hydroxy	Oxyfluorfen
Carfentrazone ethyl	Parathion methyl
Chlorfenapyr	p-Dichlorobenzene
Chlorferone (coumaphos)	Pendimethalin
Chlorothalonil	Permethrin
Chlorpyrifos	Phosmet
contrast	Piperonyl butoxide
Coumaphos	Prallethrin
Coumaphos	Pronamide
Cyfluthrin	Propiconazole
Cyhalothrin	Pyraclostrobin
Cypermethrin	Pyrethrins
Cyprodinil	Pyridaben
DDE p,p'	Pyrimethanil
Deltamethrin	Pyriproxyfen
Diazinon	Quintozene
Dicofol	Spirodiclofen
Dieldrin	Tebufenozide
Dimethomorph	Tebuthiuron
DMA (amitraz)	Tefluthrin
DMPF (amitraz)	Tetradifon
Endosulfan I	Thiabendazole
Endosulfan II	Thiacloprid
Endosulfan sulfate	Triadimefon
Esfenvalerate	Tribufos
Ethion	Trifloxystrobin
Ethofumesate	Trifluralin
Fenamidone	Vinclozolin
Fenbuconazole	
Fenhexamid	
Fenpropathrin	
Fipronil	

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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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