



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

UCT Part Number:

**ECMSSA50CT-MP** (6000 mg MgSO<sub>4</sub> and 1500 mg sodium acetate)

**CUMPSC18CT** (150 mg MgSO<sub>4</sub>, 50 mg PSA, 50 mg C18)

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

**ECMAG00D** (organic free magnesium sulfate anhydrous)

March 2012

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\*
- d) \*44% DI water, 55% acetonitrile & 1% glacial acetic acid

#### Brood and adults are extracted without using DI water

- e) Add 100 µL of an internal standard
- f) For beebread, reduce particle size by using a high speed disperser for 1 minute
- g) For comb wax melt the sample at 80° C in a water bath followed by cooling to RT
- h) Add the contents of **ECMSSA50CT-MP** pouch to the mixture

- i) Seal tube and shake vigorously for 1 minute
- j) 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  |                    |

\*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