Multiresidue Analysis in Cereal Grains Using Modified QuEChERS Method with UPLC-MS/MS and GC-TOFMS*

UCT Part Numbers

ECMSSC50CT-MP
50 mL centrifuge tube, 4 g anhydrous. magnesium sulfate, 1 g NaCl

CUMPS15C18CT
150 mg anhydrous magnesium sulfate, 150 mg PSA and 50 mg C18

Introduction:

This QuEChERS procedure is specifically developed for cereal grains (corn, oats, rice and wheat) using ultra pressure liquid chromatography-tandem mass spectrometry UPLC MS/MS and automated direct sample introduction GC-TOFMS to achieve good recoveries of over 150 analytes.

Pesticide Reference Standards

(Chemservice (West Chester, PA)

- Prepare individual pesticide stock solutions (2000 - 5000 µg/mL) in ethyl acetate or acetonitrile (MeCN) and store at -18° C
- Prepare two composite pesticide stock solutions, MIX-1 and MIX-2 at 10 µg/mL in MeCN
- Add 0.1% acetic acid to prevents degradation of base-sensitive analytes in MeCN
- See http://forums.unitedchem.com/ for complete pesticide list and mixtures

Isotopically Labeled Internal Standards

(Cambridge Isotope Laboratories, Inc. (Andover, MA))

Prepare at 5 µg/mL in acetone

- atrazine (ethylamine-d5)
- carbofuran (ring-^{13}C6)
- dimethoate (o,o-dimethyl-d6)
- 2,4-DDT (ring-^{13}C6)
- α-HCH (^{13}C6)
- parathion (diethyl-d10)

QC Working Solution

- trans-permethrin (phenoxy-^{13}C6) (1 and 5 µg/mL in acetone)

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Procedure:

1. Sample Preparation

a) Thoroughly homogenize a sample of grain products using a laboratory mill to a flourlike consistency
b) Place appropriate weight** of sample into the 50 ml centrifuge tube
c) Add 10 mL of deionized water (15 mL for rice) and 10 mL of acetonitrile
d) Add 200 µL of ISTD standard solution
e) Vortex tube to disperse sample and standard for 1 hour using a wrist action shaker
f) Add the contents of the **ECMSSC50CT-MP** pouch into the centrifuge tube
g) Immediately seal tube and vortex for 1 minute
h) Centrifuge @ rcf >3,000 for 10 minutes

2. Sample Cleanup

a) Transfer a 1 mL aliquot to a 2 mL **CUMPS15C18CT** tube
b) Vortex for 30 seconds
c) Centrifuge for 5 minutes
d) Transfer 300 µL of the supernatant into the chamber of a Mini-UniPrep syringeless filter vial (Whatman) and add 30 µL 1 µg mL QC solution*
e) Mix thoroughly
f) Transfer 125 µL of the extract in the Mini-UniPrep vial into a deactivated glass insert placed in a GC autosampler vial and cap the vial with a heat treated septum (overnight at 250° C)
g) Press the 0.2 µm polyvinylidine fluoride (PVDF) filter of the Mini-UniPrep to filter the extract for the UPLC-MS/MS analysis
h) Add 30 µL of QC standard solution
i) Sample is now ready for analysis

3. Analysis UPLC-MS/MS

UPLC-MS/MS	
Instrumentation	Acquity UPLC interfaced to a Quattro Premier triple-quad mass spectrometer (Water's Corp.) MassLynx software v 4.1 or equivalent
Column	Acquity UPLC BEH C18 (50 x 2.1 mm, 1.7 µm particle size, 130 Å pore size) or equivalent
Temperature	40°C
Injection volume	2 µL

Binary Mobile Phase & Gradient (Flowrate: 450 µL/minute):	
A	10 mM ammonium formate in water (pH 3, adjusted with formic acid)
B	10 mM ammonium formate in methanol

Time (min)	B (%)
0	30
4.0	30
7.5	60
8.5	60
10.5	100
12.5	100
12.6	30
15.0	30

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MS Conditions	
Ionization mode	ESI^+ combined with monitoring of the two most abundant MS/MS (precursor f product) ion transitions.
Extractor Voltage	4.0 V
RF Lens	0.9 V
Capillary Voltage	1.7 kV
Source Temperature	130° C
Desolvation Temperature	350° C
Collision gas	Argon
Collision gas pressure	4.31 x 10^{-3} mbar
Desolvation Gas (N_2)	Flow of 600 L/h
Cone Gas (N_2)	Flow of 100 L/h

4. For GC amenable pesticides use automated DSI-GC-TOF Mass Analyzer

a) **GC Column:** Use a combination of a 20 m x 0.25 mm id x 0.25 µm film thickness RTX-5 ms column and a 1m x 0.1 mm id x 0.1 µm film thickness RTX-pesticide 2 column (Restek). This translates into a 1.68 m x 0.1 mm id "virtual" column setting in the ATAS Evolution software or equivalent.

b) **Oven Temperature Program:** (start after a 4.5 minutes solvent vent period): 60° C, hold for 4 minutes then ramped to 180° at 20° C/minutes, then ramp 5°C/minutes to 230° C, then 20°C/minutes to 280° C, and finally ramp to 300° C at 40° C/minutes, and hold for 12 minutes. The total run time is 35 minutes.

c) **Automated DSI-GC-TOFMS Analysis**

- Agilent 6890 GC equipped with a secondary oven and nonmoving quad-jet dual stage modulator for two-dimensional comprehensive GC/GC chromatography or equivalent
- Pegasus 4D (Leco Corp., St. Joseph, MI) TOF mass spectrometer or equivalent
- Inject using CombiPAL autosampler (Leap Technologies, Carrboro, NC) or equivalent
- Automated DSI accessory (LINEX) with an Optic 3 programmable temperature vaporizer (PTV) inlet (ATAS-GL International, Veldhoven, The Netherlands) or equivalent
- Leco Chroma TOF (version 3.22) software for GC TOFMS control and data acquisition/processing or equivalent
- CombiPAL Cycle Composer with macro editor (version 1.5.2) and ATAS Evolution software (version 1.2a) to control the automated DSI process and PTV (including column flow) or equivalent

d) Automated DSI Injection

- Inject 10 µL into a disposable microvial (1.9 mm i.d., 2.5 mm o.d., 15 mm, (Scientific Instrument Services, Ringoes, NJ), Siltek deactivated (Restek Bellefonte, PA) or equivalent
- Wash with acetone heated at 250° C
- Place in a LINEX DMI tapered liner
- The liner is then transferred into the Optic inlet

e) Optic 3 PTV Conditions

- Solvent vent at an injector temperature of 100° C for 4.5 minutes
- Initial column flow of 0.8 mL/minutes and a split flow of 50 mL/minutes
- Follow by a splitless transfer of analytes for 4 minutes. The injector temperature was ramped to 280° C (at 16° C/s) Column flow changed to 1.5 mL/minutes (kept constant for the entire GC run). After the splitless period, the split flow adjusted 50 mL/minutes for 6 minutes. After 6 minutes reduce split flow to 25 mL/minutes and decrease injector temperature to 250° C

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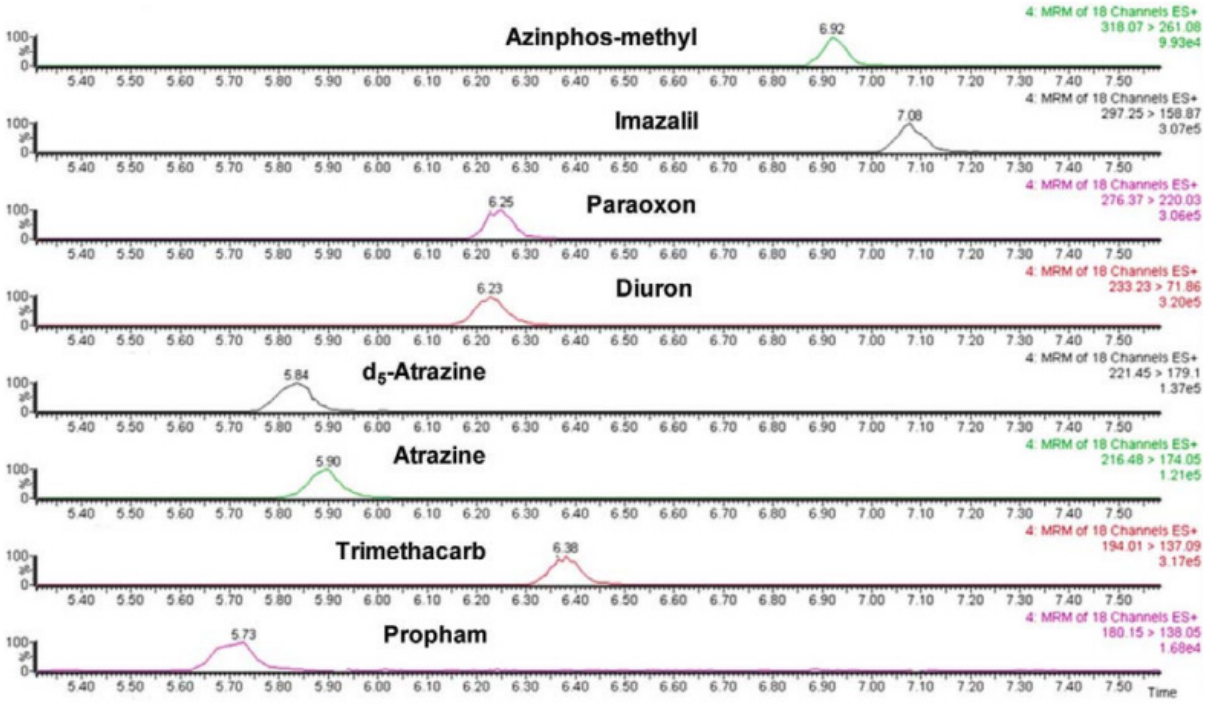
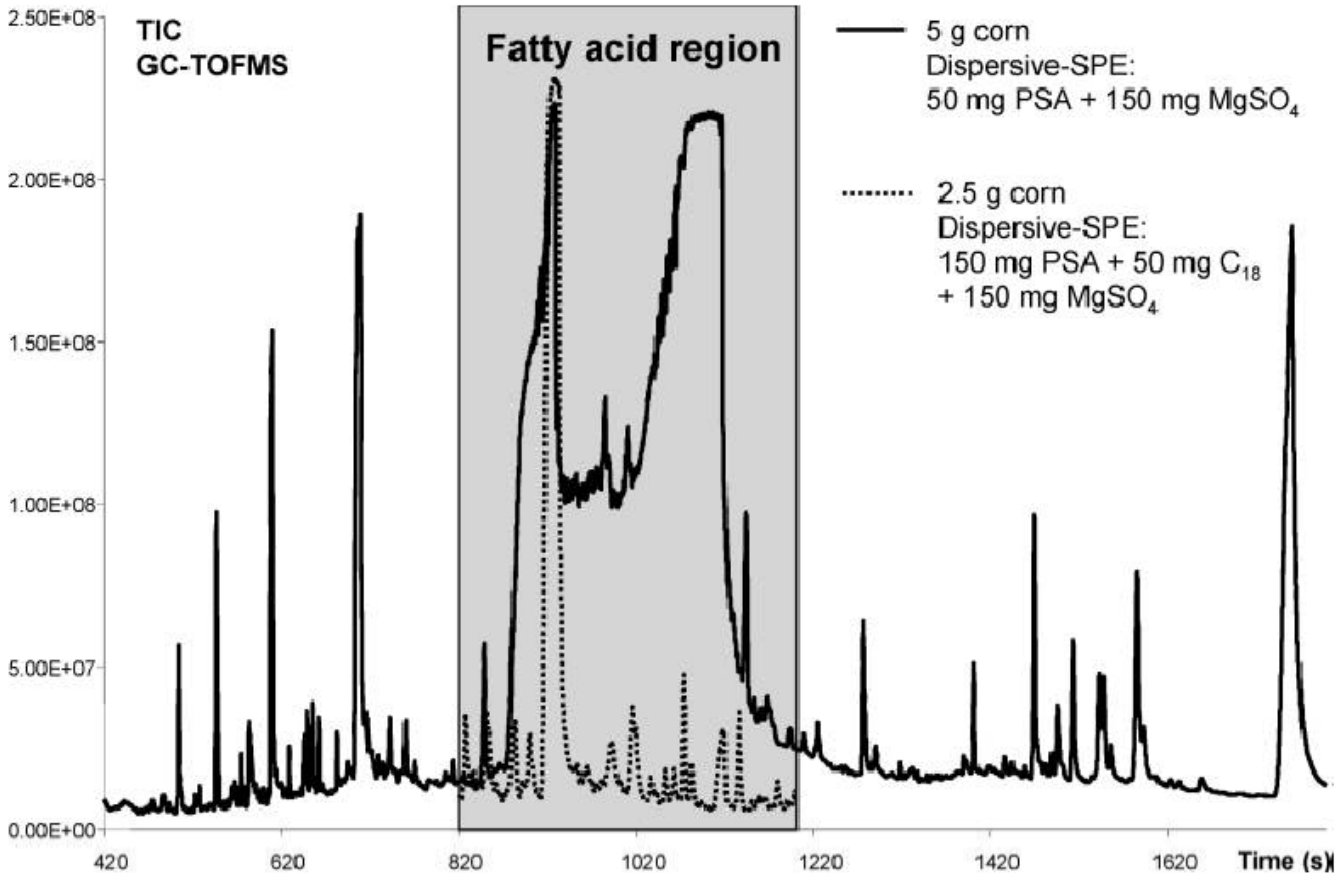
Shown Below are the UPLC-MS/MS Extracted Ion Chromatograms of Selected Pesticides
Spiked at 25 ng/g in Wheat Extract



Total Ion Chromatogram

DSI-LVI-GC-TOFMS analysis of a corn extract prepared using 5 g of sample, original QuEChERS (with 10 mL of water addition for swelling), and 50 mg of PSA in the dispersive SPE step. The highlighted region of the chromatogram is saturated with fatty acids. The dotted trace represents optimized analysis using 2.5 g of corn sample using dispersive SPE with 150mg of PSA and 50 mg of C_{18}



References:

[1] *Summarized from Mastovska et al, "Pesticide Multiresidue Analysis in Cereal Grains Using Modified QuEChERS Method Combined with Automated Direct Sample Introduction GC-TOFMS and UPLC-MS/MS Techniques" ," J of Agricultural and Food Chemistry, Full article may be found at http://forums.unitedchem.com/

[2] ** Corn 2.5 g, oat 3.5 g, rice 5.0 g, wheat 5.0 g

Listing of chemical suppliers and instrument manufacturers does not constitute endorsement by UCT.

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