Laboratories Division, Georgia Department of Agriculture	**Title: The QuEChERS Method of Sample Preparation for Pesticide Residues Analysis**	Page 1 of 7

Issued by:	**Date Issued:**	Revision No.: 6
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(initials) (title)	**P-614.6**	

I. **PURPOSE-** This document details the QuEChERS method of sample extraction and cleanup. It also references the instrument conditions for analyzing samples prepared by this protocol.

II. **SCOPE-**

A. *Pesticides-* most compounds which can be detected by gas chromatography/mass spectrometry, as well as carbamates, substituted ureas, and cyclohexanediones

B. *Sample Matrices-* any plant tissue, soil of any type, and feeds

III. **RESPONSIBILITY-** Any person in the Atlanta or Tifton Pesticide Residues Laboratories who has been trained in this method may prepare and/or analyze samples. It is the responsibility of the Chemical Laboratories Manager to ensure that staff adhere(s) to this procedure.

IV. **REFERENCES-**

A. The Georgia Department of Agriculture Chemical Hygiene Plan

B. *Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Approach for the Determination of Pesticide Residues* (presentation by Steven J. Lehotay at the 38th annual Florida Pesticide Residues Workshop)

C. *Evaluation of the QuEChERS Method* (Frank J Schenck and James E. Hobbs, LIB #4293, volume 18, number 11)

D. *New Developments in the "Quick, Easy, Cheap, Effective, Rugged, and Safe" (QuEChERS) Method for Pesticide Residues in Food*

(presentation by Dr. Katerina Mastovska at the 41st annual Florida Pesticide Residues Workshop)

E. P-1005 (Mass Measurement)

F. P-604 (The Preparation of Pesticide Standard Solutions)

G. P-600 (The Numbering of Pesticide Standards)

V. RELATED DOCUMENTS-

VI. DEFINITIONS-

A. *Matrix Blank-* a subsample of material which is known to be free of the compounds for which a sample batch is being screened. The matrix composition should be representative of the types of samples in the batch.

B. *Matrix Control-* a spiked subsample of the Matrix Blank material.

C. *Matrix Standard-* a solution consisting of a known amount of analyte in extract of Matrix Blank.

D. *Reagent Blank-* consists of everything used in the preparation of a sample, except actual matrix.

E. *Sample Batch-* a group of samples which are prepared together.

VII. SAFETY EQUIPMENT REQUIRED-

A. **Laboratory Coat** (worn at all times)

B. **Safety Glasses/Goggles** (worn at all times)

C. **Disposable Latex Gloves** (powder-free, worn at all times during sample preparation)

D. **Fume Hood**

E. **Flammables Storage Cabinet** (used for storing acetonitrile)

VIII. CHEMICALS REQUIRED-

A. Acetonitrile (residues grade)

B. Acetic Acid (glacial)

C. Water (18 megohm)

D. Pesticide Standards (as needed, for spiking of Control and for preparation of matrix standard solutions)

E. Magnesium Sulfate (anhydrous powder, at least 99% purity)

F. Primary Secondary Amine solid phase packing

G. Sodium Acetate (anhydrous granular, at least 99.0% purity)

IX. EQUIPMENT REQUIRED-

A. Robot Coupe food processor (or equivalent), with metal chopping/pureeing blade

B. Coffee Grinder: Braun Aromatic or equivalent

C. Balance Weight, 10 g (Class S)

D. Analytical Balance (capable of weighing to at least 2 decimal places)

E. Centrifuge Tubes, 50 mL capacity (Teflon with PTFE-lined screwcap, or glass with gg stopper or PTFE-lined screwcap)

F. Floor Centrifuge (Beckman GPK or equivalent, capable of up to 3,870 rpm)

G. Benchtop Centrifuge (for the Atlanta Laboratory protocol), capable of up to 3000 rpm)

H. Scoopulas (or equivalent)

I. Vortexer (Fisher Touch Mixer Model 231, or equivalent)

J. Centrifuge Tubes, 15 mL capacity (glass, with PTFE-lined screwcap or gg stopper, and graduated in 0.1 mL increments)

K. Weighing Funnels (size)

L. Beakers, 10 mL (glass)

M. Pipettor, Eppendorf or equivalent (10 µL to 100 µL tip capacity)

N. Pipet Bulb (rubber) for Pasteur pipets

O. Freezer (secured)

X. CONSUMABLES REQUIRED-

A. Tips for the pipettor

B. Pasteur Pipets (borosilicate glass, 5 ¾", disposable)

C. Syringe filters (Luer-loc, 15 mL diameter, PTFE membrane with 0.2 μm pore diameter)

D. Syringes (disposable, plastic, 3 cc)

E. Autosampler Vials and Caps

XI. INSTRUMENTATION REQUIRED-

A. Gas Chromatograph: HP5890 or Agilent 6890 or equivalent, with autosampler

B. Mass Selective Detector: HP5970 or Agilent 5973 or equivalent

C. LC/MS System (Agilent 1100 HPLC system or equivalent, plus Agilent G1946C mass selective detector or equivalent, with API-ES and APCI Chambers)

D. Chem Stations for the above systems

XII. METHODOLOGY-

A. *Subbing and Homogenizing-*

1. Mix the entire sample by hand. Be sure to turn it over, so that the bottom material will be mixed in as well.
2. Draw at random enough matrix to fill about 2/3 of the Robot Coupe's reservoir.
3. Chop and blend until no smaller particle-size can be obtained. If the matrix is not fine enough for extraction:

   a) Remove the reservoir and take out its blade.

   b) Mix contents by hand.

c) Introduce enough sample into the coffee grinder's reservoir to fill about 2/3 of it.

d) Grind subsample while shaking device, until no finer matter size can be obtained.

e) Using a scoopula, mix the contents of the reservoir.

B. *Subsample Extraction and Cleanup-*

1. Weigh 10 g sample into a 50 mL centrifuge tube.

2. *For matrices other than ground feeds:* Add 10 mL acetonitrile extraction solvent (contains 0.1% (v/v) acetic acid)*. If it is completely absorbed by the subsample, add 10 mL water. If the solution is still completely absorbed by the subsample, add 8 mL water.

OR pH 5.5

*If the Control recovery for a target compound is unacceptably low, the compound may not remain stable in the 0.1% acidic solution. Extract a new subsample (and Control and Matrix Blank), using 1% (v/v) acetic acid in acetonitrile.

3. *For ground feeds:* Add 10 mL water first, followed by the extraction solvent. Then, proceed as in Step 2.

4. Add 1 g sodium acetate, *then* 4 g magnesium sulfate.

5. Immediately vortex or shake by hand for 90 seconds.

6. Centrifuge for 10 minutes:

   a) In the Atlanta Lab, use a numerical setting of "45".

   b) In the Tifton Lab, use a speed setting of 3,870 rpm.

7. Transfer 2.0 mL or 4.0 mL solution to a 15 mL centrifuge tube.

8. Add PSA and magnesium sulfate:

a) For 2.0 mL solution, add 100 mg PSA and 300 mg magnesium sulfate.

b) For 4.0 mL solution, add 200 mg PSA and 600 mg magnesium sulfate.

9. Shake/vortex for 30 seconds.

10. Centrifuge for 5 minutes:

a) In the Atlanta Lab, use a numerical setting of "3" for the benchtop centrifuge.

b) In the Tifton Lab, use a speed setting of 3,000 rpm for the floor centrifuge.

11. Filter ½ to 1 mL of the extract into an autosampler vial, or use the mini 0.2 µ filter vials

XIII. INSTRUMENT PARAMETERS AND EXPECTED SPECTRA-

This information can be found in the "GC/MS Methods" binder, or the "LC/MS Method binders, or the "LC/Triple Quad" binders

XIV. QUALITY CONTROL-

A. *Balance measurements* must be traceable to NIST mass standards.

B. A *Matrix Control* and a *Matrix Blank* must be prepared at the same time as the sample or batch of samples.

C. If the analytes of interest are known for the samples in the batch, the Control is spiked with each of these.

D. If the analytes of interest are not known, the Control is spiked with representatives of the classes which might be relevant to the case. For instance, if an animal has gotten sick, and the symptoms displayed are those of cholinesterase inhibitor poisoning, then it is logical to spike the Control with at least one organophosphate and one carbamate known from previous cases to have been used as animal poisons.

The target concentration(s) of the spiked compounds will depend on what concentration(s) might be expected in the samples themselves. For example, in the case of a drift complaint, we would expect usually to see less than 1 ppm of analyte, if present.

So, a spike level of 0.2 ppm might be appropriate.

E. A *Reagent Blank* is prepared at the same time.

F. *Two matrix standards* are prepared, for quantitation, for determination of the Limit(s) of Detection, and for monitoring the condition of the analytical instrument during a run sequence. One solution will contain the analyte(s) in the concentration(s) expected in the Control's extract, and the concentration(s) in the other will be near the Instrument Limit of Detection, if known for the compound(s).

XV. RECORDS GENERATED-

A. Balance calibration and instrument maintenance records

B. Chain-of-custody information

C. Batch Worksheet(s) with sample preparation and Control and Matrix Blank information.

Printing Time: 3:00:31 PM
Printing Date: Tuesday, November 13, 2012

"Regular" QuEChERS

Acquisition Information:

Acquisition Method:	CP_Targeted_Screen.dam
Created:	Friday June 01 2012 15: 27: 00 PM
Last Modified:	Monday June 11 2012 11: 05: 31 AM
Comment:	
Synchronization Mode:	LC Sync
Auto-Equilibration:	On
Auto-Equilibration Duration (min):	0.00
Acquisition Duration:	20min5sec
Number Of Scans:	1205
Periods In File:	1
Acquisition Module:	Acquisition Method
Software version	Analyst 1.6

Period 1:

Scans in Period:	1205
Relative Start Time:	0.00 msec
Experiments in Period:	1

Period 1 Experiment 1:

Scan Type:	MRM (MRM)
Scheduled MRM:	Yes
Polarity:	Positive
Scan Mode:	N/A
Ion Source:	Turbo Spray
MRM detection window:	120 sec
Target Scan Time:	1.0000 sec
Resolution Q1:	Unit
Resolution Q3:	Unit
Intensity Thres.:	0.00 cps
Settling Time:	0.0000 msec
MR Pause:	5.0070 msec
MCA:	No
Step Size:	0.00 Da

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
207.100	132.100	1.46	DP	61.00	61.00	Aldicarb Sulfoxide 1
			CE	10.00	10.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
207.100	89.100	1.46	DP	61.00	61.00	Aldicarb Sulfoxide 2
			CE	19.00	19.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
223.100	86.100	1.71	DP	70.00	70.00	Aldicarb Sulfone 1
			CE	20.00	20.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
223.100	148.000	1.71	DP	70.00	70.00	Aldicarb Sulfone 2
			CE	12.00	12.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
237.100	72.100	1.90	DP	63.00	63.00	Oxamyl 1
			CE	25.00	25.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
237.100	90.100	1.90	DP	63.00	63.00	Oxamyl 2
			CE	11.00	11.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
163.000	88.000	2.26	DP	70.00	70.00	Methamyl 1
			CE	15.00	15.00	
			CXP	10.00	10.00	

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Electronic Signature: no
Operator: Administrator

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Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
163.000	106.000	2.26	DP	56.00	56.00	Methomyl 2
			CE	13.00	13.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
238.000	163.000	3.75	DP	86.00	86.00	3-OH Carbofuran 1
			CE	21.00	21.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
238.000	181.000	3.75	DP	66.00	66.00	3-OH Carbofuran 2
			CE	15.00	15.00	
			CXP	10.00	10.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
208.200	116.100	4.63	DP	51.00	51.00	Aldicarb 1
			CE	11.00	11.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
208.200	89.000	4.63	DP	51.00	51.00	Aldicarb 2
			CE	20.00	20.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
210.100	111.000	5.49	DP	61.00	61.00	Propoxur 1
			CE	19.00	19.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
210.100	168.100	5.49	DP	61.00	61.00	Propoxur 2
			CE	11.00	11.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
222.200	123.100	5.57	DP	66.00	66.00	Carbofuran 1
			CE	29.00	29.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
222.200	77.100	5.57	DP	66.00	66.00	Carbofuran 2
			CE	60.00	60.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
202.100	145.000	5.86	DP	86.00	86.00	Carbaryl 1
			CE	15.00	15.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
202.100	127.000	5.86	DP	86.00	86.00	Carbaryl 2
			CE	39.00	39.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
355.000	88.000	6.35	DP	70.00	70.00	Thiodicarb 1
			CE	29.00	29.00	
			CXP	6.00	6.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
355.000	108.000	6.35	DP	41.00	41.00	Thiodicarb 2
			CE	25.00	25.00	
			CXP	4.00	4.00	
Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
233.000	72.000	6.57	DP	76.00	76.00	Diuron 1
			CE	33.00	33.00	
			CXP	4.00	4.00	

Collected by: N/A
Electronic Signature: no
Operator: Administrator

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Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
233.000	46.000	6.57	DP	96.00	96.00	Diuron 2
			CE	37.00	37.00	
			CXP	8.00	8.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
249.000	160.000	7.08	DP	81.00	81.00	Linuron 1
			CE	23.00	23.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
249.000	182.000	7.08	DP	41.00	41.00	Linuron 2
			CE	49.00	49.00	
			CXP	6.00	6.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
311.000	158.000	7.93	DP	66.00	66.00	Diflubenzuron 1
			CE	19.00	19.00	
			CXP	4.00	4.00	

Q1 Mass (Da)	Q3 Mass (Da)	Time (min)	Param	Start	Stop	ID
311.000	141.000	7.93	DP	36.00	36.00	Diflubenzuron 2
			CE	25.00	25.00	
			CXP	6.00	6.00	

Parameter Table(Period 1 Experiment 1):

CUR:	35.00
CAD:	High
IS:	5500.00
TEM:	550.00
GS1:	50.00
GS2:	50.00
EP	10.00

Valco Valve Diverter

	Total Time (min)	Position
1	0.1	Mass Spec
2	14.0	Waste

Shimadzu LC Method Properties
Shimadzu LC system Equilibration time = 0.00 min
Shimadzu LC system Injection Volume = 5.00 ul
Shimadzu LC Method Parameters
Pumps
Pump A Model: LC-20ADXR
Pump B Model: LC-20ADXR
Pumping Mode: Binary Flow
Total Flow: 0.2500 mL/min.
Pump B Conc: 20.0 %
B Curve: 0
Pressure Range (Pump A/B): 0 - 5796 psi
Autosampler
Model: SIL-20ACXR
Rinsing Volume: 200 uL
Needle Stroke: 52 mm.
Rinsing Speed: 35 uL/sec.
Sampling Speed: 5.0 uL/sec.
Purge Time: 25.0 min.
Rinse Dip Time: 0 sec.
Rinse Mode: No rinsing
Cooler Enabled: Yes
Cooler Temperature: 15 deg. C
Control Vial Needle Stroke: 52 mm
Oven
Model: CTO-20A
Temperature Control: Enabled
Temperature: 40 deg. C
Max. Temperature: 90 deg. C
System Controller
Model: CBM-20A
Power: On
Event 1: Off

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Electronic Signature: no
Operator: Administrator

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Event 2: Off
Event 3: Off
Event 4: Off
Solenoid Valve

Pump A (FCV-11AL)
Port 1 Valve Position: A Port 2 Valve Position: A Port 3 Valve Position: A Time Program

Time	Module	Events	Parameter
0.10	Pumps	Pump B Conc.	20
8.00	Pumps	Pump B Conc.	90
14.00	Pumps	Pump B Conc.	90
15.00	Pumps	Pump B Conc.	20
20.00	Pumps	Pump B Conc.	20
20.10	System Controller	Stop	

Collected by: N/A
Electronic Signature: no
Operator: Administrator