United States Department of Agriculture

Food Safety and Inspection Service

CLG-PST5.11

Screening for Pesticides by UHPLC-MS/MS and GC-MS/MS

This method is the laboratory procedure for screening 106 pesticides from 10 pesticide classes in muscle tissue from 7 species (bovine, caprine, equine, ovine, porcine, poultry, fish of the order *Siluriformes* (catfish)), liquid egg products, and powdered egg.

Executive Summary

This is a multi-residue method that is used to screen 106 pesticides from 10 pesticides classes (carbamates, conazoles/triazoles, halogenated, neonicotinoids, organochlorines, organophosphates, general pesticides, pyrethroids, substituted benzenes, and triazines) in muscle tissues from 7 animal species, liquid egg products, and powdered egg products. One of the method's key features is its ability to be used as a high-throughput screening method for various pesticide residues which include both volatile and non-volatile pesticides. The minimum levels of applicability (MLA) or lowest levels at which an FSIS method has been successfully validated for a residue in each matrix for this method are listed in Table 16.

Notice of Change

Pyrethrin I and II were previously listed in the method as the monitored residues for natural pyrethrins. Natural pyrethrins have a residue tolerance definition listed in 40 CFR 180, which does not include Pyrethrin II. Therefore, Pyrethrin II was removed from the method.

There is currently no commercially available reference material or analytical standard for Ethion monoxon. Therefore, Ethion monoxon was removed as an analyte of interest from the method. The commercially available parent compound, Ethion, will remain in the method.

In addition to these method changes, an executive summary and safety hazards section has been added to method.

No changes were made to the flow chart found in CLG-PST5 Appendix 1.

Table of Contents

Safety Precautions

The personnel performing the analysis must read the Safety Data Sheets (SDS) for the standards and reagents used in this method. The hazards and recommended safe procedures for use are listed in Table 17. Follow applicable federal, state, and local regulations regarding the disposal of chemicals listed in this method.

Introduction

Pesticides are used in agriculture to prevent, mitigate, destroy, or repel pests. The Environmental Protection Agency (EPA) regulates the approval and use of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act. The EPA establishes and publishes regulations setting tolerances for residues of pesticides. The National Residue Program (NRP) is an interagency program that is designed to identify, prioritize, and analyze residues in meat, poultry, and egg products. The Food Safety and Inspection Service (FSIS) administers the NRP by collecting and testing samples of domestic and imported meat (including *Siluriformes* fish products), poultry, and egg products for pesticides to verify that these products are below tolerances and are safe, wholesome, and accurately labeled. FSIS publishes an Annual Sampling Plan to provide information on the process of sampling meat, poultry, and egg products for pesticides of public health concern. The NRP is monitored and modified annually to set priorities based on data analyses that identify trends in detected residues.

Method Overview

CLG-PST5 is used for screening of 106 pesticides in 10 pesticides classes. The number of pesticides in those classes are listed as follows: 9 carbamates, 4 conazoles/triazoles, 9 halogenated, 4 neonicotinoids, 20 organochlorines, 18 organophosphates, 28 general pesticides, 8 pyrethroids, 3 substituted benzenes, and 3 triazines. The method is applicable for analysis of pesticides in bovine, caprine, ovine, poultry, porcine, equine, and *Siluriformes* (catfish), liquid egg products, and powdered egg.

KEY DEFINITIONS

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe): A solid phase extraction method for detection of pesticide residues in food.

Protein-precipitation: An extraction technique resulting in solid material being left at the bottom of an extraction vessel with the extract or liquid layer containing the analyte. The liquid layer can be separated out for further analysis.

Solid Phase Extraction (SPE): An extraction technique that utilizes a solid support that contains an adsorbing surface or chemical coating that can interact with analyte.

UHPLC-MS/MS: An analytical technique where there is a physical separation of target compounds followed by their mass-based detection.

GC-MS/MS: An analytical technique that involves separation analytes with gas chromatography followed by analysis of masses of individual atoms or molecules through mass spectrometry.

Pesticide residues are extracted from muscle tissue through an extraction with ethyl acetate and QuEChERS salts containing sodium chloride and magnesium sulfate. The crude material is separated through filtration, resulting in an ethyl acetate extract. A solvent exchange is conducted with acetonitrile, which is then followed by a clean-up through precipitation using ultra-low temperature freezing to separate the fats from the samples. The liquid layer is transferred, and solid-phase extraction (SPE) is then used to further clean up the extracts. The extracts are separated for liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) analysis (69 pesticides) and for gas chromatography with tandem mass spectrometry (GC-MS/MS) analysis (37 pesticides). UHPLC-MS/MS is used for analysis of water-soluble compounds, while GC-MS/MS is used for analysis of volatile compounds. Prior to UHPLC-MS/MS analysis, the extract is further cleaned up with dispersive SPE and QuEChERS salts containing an anion exchange sorbent. Prior to GC-MS/MS analysis, the extract is further cleaned up with anion exchange SPE and a solvent exchange with toluene.

Both the UPLC-MS/MS and GC-MS/MS instrumental analyses are independent of one another. In the case that an instrument is not available, samples can continue to go through the respective extraction and clean-up processes with the results for the available system being reported while excluding the results for unavailable instrument. Additionally, this method may be performed

using standards or solutions that contain fewer analytes than the method applicability. When that occurs, the excluded analytes would not be included in the reported results.

This method is to be performed using the standards/solutions for the respective analyte(s) of interest. Only applicable standards/solutions are necessary for reporting results.

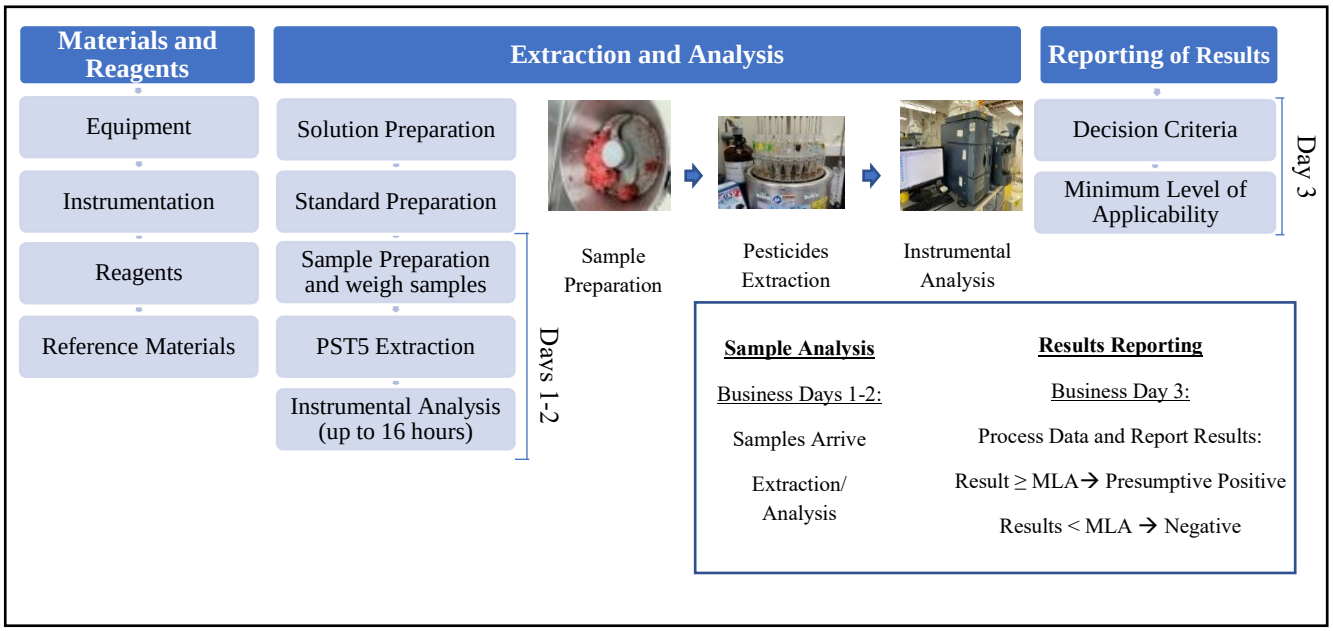

Figure 1: Overview and timeframe of Pesticides (CLG-PST5). Materials and reagents are obtained and utilized to prepare solutions and standards. The samples arrive at laboratory, are prepared into a homogenized mixture, weighed, extracted, and analyzed by UHPLC-MS/MS and GC-MS/MS on business days 1-2. Screening results are reported on business day 3. This figure represents the best-case scenarios, but analyses may take longer. Photos courtesy of Hue Quach, USDA-FSIS and Ryan Matsuda, USDA-FSIS.

Decision Criteria

A sample is considered negative if the results are less than the minimum level of applicability (MLA). A sample is considered a presumptive positive if the results are greater than or equal to the MLA. Presumptive positive results will require further analysis through additional methods.

KEY DEFINITIONS

MLA: Lowest level at which an FSIS method has been successfully validated for a residue in each matrix. Full definition is on the CLG website.

Disclosure Statement

FSIS does not specifically endorse any test products listed in this method. FSIS acknowledges that equivalent equipment, reagents, or solutions may be suitable for laboratory use. The FSIS laboratory system uses method performance requirements when evaluating the equivalence of an alternative equipment, reagent, or solution for a given analyte and sample matrix pair. Significant equivalence changes would require FSIS laboratory leadership approval.

Materials and Reagents

Equipment

Table 1: Equipment Required to Perform CLG-PST5

Equipment	Supplier and Part Number	Purpose
Food Processor	Robot Coupe USA, Inc	Homogenize sample
Top Loading Balance	General lab supplier	Record weight of standard reagent. Minimum accuracy Minimum accuracy ±0.01 g
Microcentrifuge	General lab supplier	Separates the solid sample material from the extraction solution
Centrifuge	General lab supplier	Separates the solid sample material from the extraction solution Capable of centrifuging 50 mL glass test tubes
Centrifuge tubes, Polypropylene (PP), 50 mL	SARSTEDT, 62.548.101	Contain sample material and extraction vessel
Centrifuge tubes, glass, 50 mL	Pyrex, 8084-50	Contain extraction solution and extraction vessel, Pyrex tube with stopper
Centrifuge tubes, glass, 15 mL	Kimble Chase, 45166-15	Contain extraction solution and extraction vessel, Pyrex tube with stopper
Shaker	General lab supplier	Facilitates extraction of pesticides from the sample
Multi Tube Vortex	General lab supplier	Facilitates extraction of pesticides from the sample
Freezer, -10 °C, -20 °C, and -70 °C	General lab supplier	Storage of standards and reagents
Nitrogen Evaporator Apparatus with Heated Water Bath	General lab supplier	Reduces extraction solution down to desired volume
Positive Pressure Manifold	UCT, LLC, VMFPPM16	Perform SPE Clean Up
1000 mg C18 SPE Columns	UCT, LLC, CEC181M6	Separates out the pesticides from the extraction solution

Fluted filter paper	VWR, 28333-043	Filter extracts
Nylon Syringe Filter, 0.2 µm	Pall Corporation, 4561	Filter extracts
500 mg PSA (Primary Secondary Amine) SPE Columns	UCT, LLC, CUPSA156	Clean up sample for GC-MS/MS analysis
Micro centrifuge tubes with QuEChERS salts (150 mg $MgSO_4$ & 50 mg PSA)	UCT, LLC, CUMPS2CT	Clean up sample for LC-MS/MS analysis
Bottle-Top Dispensers, 1 mL to 5 mL, 2 mL and 10 mL	General lab supplier	Adds solutions.
Repeating pipettes and tips, 2 µL - 20 µL, 20 µL to 1000 µL, 500 µL to 2500 µL	General lab supplier	Dispense standards and reagents.
Disposable Pipettes	General lab supplier	Dispense standards and reagents
Disposable Transfer Pipettes	General lab supplier	Dispense standards and reagents
Syringe, Plastic, 3 mL	Becton Dickenson, 309657	Filter final extracts
Auto Sampler Vials	General lab supplier	Store final extractions for analysis
Glassware, Class A	General lab supplier	Measuring standards and reagents
LC Vial Caps	Supelco, 29049-U	Cap autosampler vials for LC analysis
GC Vial Caps	Agilent, 5185-5820	Cap autosampler vials for GC analysis
Disposable Glass Tubes, 16×12 mm	VWR, 47729-578	Storage of extract

Instrumentation

Table 2: Instrumentation

Instrument	Supplier and Model Number	Purpose
Waters UPLC-MS/MS System	Waters Xevo I-Class LC, Waters Xevo TQ-S micro Mass Spectrometer	Extract analysis
Waters UPLC HSS T3, 2.1 × 100 mm, 1.8 µm	Waters, 186003539	Extract analysis
Waters VanGuard Pre-column UPLC HSS T3, 2.1 × 5.0 mm, 1.8 µm	Waters, 186003976	Extract analysis
Gas Chromatograph	Agilent, 7890B	Extract analysis
GC Quadrupole Mass Spectrometer	Agilent, 7010	Extract analysis
Agilent, J&W HP-5MS Ultra Inert 15 m, 0.25 mm, 0.25 µm 7 in cage, two columns are used in series	Agilent, 19091S-431UI	Extract analysis

Reagents

Table 3: Reagents

Reagent	Supplier and Part Number
QuEChERS Salts Packets (8 g Magnesium Sulfate ($MgSO_4$) & 2 g Sodium Chloride (NaCl))	UCT, LLC, ECQUVIN50CT-MP
Magnesium sulfate ($MgSO_4$), anhydrous	General lab supplier
Acetic acid – ACS Grade	General lab supplier
Ethyl acetate – LC Grade	General lab supplier
Acetonitrile – LC Grade	General lab supplier
Acetone – LC Grade	General lab supplier
Toluene – LC Grade	General lab supplier
Methanol – LC Grade	General lab supplier
Ammonium Acetate	General lab supplier
Formic Acid – ACS Grade	General lab supplier
Water – Resistivity of > 18 MΩ-cm	House system
Isopropanol – LC-MS Grade	General lab supplier

Reference Materials

Table 4: Reference Materials

Standard	Supplier	Catalog Number
Custom Pesticide Standard (Internal standard Trichloronate and Ethoprophos	Accustandard	S-23242
Custom Pesticide Standard	Accustandard	S-66100-01
Custom Pesticide Standard	Accustandard	S-66100-02
Custom Pesticide Standard	Accustandard	S-63904-01-R1
Custom Pesticide Standard	Accustandard	S-63904-02
Custom Pesticide Standard	Accustandard	S-63903-R1-SET

Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards are to be assigned an expiration date that is no later than the stability stated in the method.

Extraction and Analysis

Solution Preparation

Table 5: Preparation of Solutions

Solution	Procedure
1% Acetic acid in acetonitrile (v/v)	1) Using a graduated cylinder measure 1980 mL of acetonitrile. 2) Using a graduated cylinder measure 20 mL of acetic acid. 3) Combine and mix in a 2 L glass storage container for use. 4) Store at room temperature. *Solution expires 1 year after preparation.*
3:1 Acetone/Toluene (v/v)	1) Measure 500 mL of toluene and add to a 2 L graduated cylinder. 2) Measure 1500 mL of acetone and add to same cylinder. 3) Mix well for use. 4) Store at room temperature. *Solution expires 1 year after preparation.*
LC-MS/MS Mobile Phase A (5 mM ammonium acetate in 0.1% formic acid in water (v/v))	1) Measure 0.771 g ammonium acetate. 2) Dissolve and transfer the ammonium acetate to a 2 L graduated cylinder or volumetric flask with water. 3) Measure and add 2 mL of formic acid to same cylinder or volumetric flask. 4) Dilute to volume with water. 5) Mix well and transfer to a glass storage container for use. 6) Store at room temperature. *Solution expires 1 year after preparation.*
LC-MS/MS Mobile Phase B (0.1% formic acid in methanol (v/v))	1) Measure 2 mL of formic acid and add to a 2 L graduated cylinder or volumetric flask. 2) Dilute to volume with methanol. 3) Mix well and transfer to glass storage container for use. 4) Store at room temperature. *Solution expires 1 year after preparation.*
LC-MS/MS Weak Wash (10% methanol in water by volume)	1) Measure 100 mL of methanol and add to a 1 L glass storage container. 2) Measure 900 mL of water and add to same container. 3) Mix well for use. 4) Store at room temperature *Solution expires 1 year after preparation.*

LC-MS/MS Strong Wash (0.5% formic acid in 1:1:1:1 acetonitrile: methanol: isopropanol: water)	1) Measure 250 mL of acetonitrile and add to a 1 L glass storage container. 2) Measure 250 mL of methanol and add to same container. 3) Measure 250 mL of isopropanol and add to same container. 4) Measure 250 mL of water and add to same container. 5) Measure 5.0 mL formic acid and add to same container. 6) Mix well for use. 7) Store at room temperature. *Solution expires 1 year after preparation.*

Standard Preparation

Table 6: LC Mixed Pesticide Standard

Cmpd #	Pesticide	CAS #	Stock Conc. (µg/mL ethyl acetate)	Spiking Solution Conc. (µg/mL ethyl acetate)
1	3-Hydroxycarbofuran	16655-82-6	10	1
2	Acephate	30560-19-1	20	2
3	Acetamiprid	135410-20-7	10	1
4	Alachlor	15972-60-8	10	1
5	Aldicarb	116-06-3	20	2
6	Aldicarb sulfone	1646-88-4	20	2
7	Aldicarb sulfoxide	1646-87-3	50	5
8	Atrazine	1912-24-9	20	2
9	Azinphos methyl	86-50-0	20	2
10	Azoxystrobin	131860-33-8	10	1
11	Benoxacor	98730-04-02	10	1
12	Boscalid	188425-85-6	30	3
13	Buprofezin	69327-76-0	50	5
14	Carbaryl	63-25-2	50	5
15	Carbofuran	1563-66-2	10	1
16	Carfentrazone ethyl	128639-02-1	10	1
17	Clothianidin	210880-92-5	20	2
18	Coumaphos O	321-54-0	20	2
19	Coumaphos S	56-72-4	20	2
20	Desethylatrazine	6190-65-4	20	2
21	Diazinon	333-41-5	10	1
22	Dichlorvos (DDVP)	62-73-7	20	2
23	Difenoconazole	119446	30	3
24	Diflubenzuron	35367-38-5	25	2.5
25	Dimethoate	60-51-5	20	2

Cmpd #	Pesticide	CAS #	Stock Conc. (µg/mL ethyl acetate)	Spiking Solution Conc. (µg/mL ethyl acetate)
26	Diuron	330-54-1	160	16
27	Ethion	563-12-12	20	2
28	Ethofumesate	26225-79-6	40	4
29	Fenoxaprop ethyl	66441-23-4	20	2
30	Fluridone	59756-60-4	50	5
31	Fluroxypyr-1-Methylheptyl-Ester	81406-37-3	10	1
32	Fluvalinate	102851-06-9	15	1.5
33	Hexazinone	51235-04-2	60	6
34	Hexythiazox	78587-05-0	20	2
35	Imazalil	35554-44-0	10	1
36	Imidacloprid	138261-41-3	50	5
37	Indoxacarb	144171-61-9	50	5
38	Linuron	330-55-2	50	5
39	Malathion	121-75-5	80	8
40	Metalaxyl	57837-19-1	20	2
41	Methamidophos	10265-92-6	20	2
42	Methomyl	16752-77-5	60	6
43	Methoxyfenozide	161050-58-4	10	1
44	Metribuzin	21087-64-9	100	10
45	Myclobutanil	88671-89-0	20	2
46	Norflurazon	27314-13-2	20	2
47	Omethoate	1113-02-6	20	2
48	Piperonyl butoxide	51-03-6	45	4.5
49	Pirimiphos methyl	29232-93-7	20	2
50	Prallethrin	23031-36-9	80	8
51	Profenofos	41198-08-7	20	2
52	Propachlor	1918-16-7	20	2
53	Propanil	709-98-8	50	5
54	Propetamphos	31218-83-4	15	1.5
55	Propiconazole	60207-90-1	30	3
56	Pyraclostrobin	175013-18-0	100	10
57	Pyrethrin I	8003-34-7	56	5.6
58	Pyridaben	96489-71-3	18	1.8
59	Pyriproxyfen	95737-68-1	40	4
60	Resmethrin (cis& trans)	10453-86-8	100	10
61	Simazine	122-34-9	20	2
62	Sulprofos	34500-43-2	50	5
63	Tebufenozide	112410-23-8	80	8

Cmpd #	Pesticide	CAS #	Stock Conc. (µg/mL ethyl acetate)	Spiking Solution Conc. (µg/mL ethyl acetate)
64	Tetrachlorvinphos	22248-79-9	20	2
65	Tetraconazole	11281-77-3	10	1
66	Thiabendazole	148-79-8	30	3
67	Thiamethoxam	153719-23-4	20	2
68	Thiobencarb	28249-77-6	100	10
69	Trifloxystrobin	141517-21-7	10	1

Table 7: GC Mixed Pesticide Standard

Cmpd #	Pesticide	CAS #	Stock Conc. (µg/mL ethyl acetate)	Spiking Solution Conc. (µg/mL ethyl acetate)
1	1-Naphthol	90-15-3	60	6
2	Aldrin	309-00-2	50	5
3	Bifenthrin	82657-04-3	10	1
4	Chlordane cis	5103-71-9	20	2
5	Chlordane trans	5103-74-2	20	2
6	Chloroneb	2675-77-6	18	1.8
7	Chlorothalonil	1897-45-6	120	12
8	Chlorpropham	101-21-3	60	6
9	Chlorpyrifos	2921-88-2	15	1.5
10	Chlorpyrifos methyl	5598-13-0	10	1
11	DDD o,p'	53-19-0	100	10
12	DDD p,p' + DDT o,p'	72-54-8 & 789-02-6	100+100	10+10
13	DDE o,p'	3424-82-6	100	10
14	DDE p,p'	72-55-9	100	10
15	DDT p,p'	50-29-3	100	10
16	Dieldrin	60-57-1	50	5
17	Endosulfan I	959-98-8	100	10
18	Endosulfan II	33213-65-9	100	10
19	Endosulfan sulfate	1031-07-8	100	10
20	Fenpropathrin	39515-41-8	50	5
21	Fipronil	120068-37-3	10	1
22	Fipronil desulfinyl	205650-65-3	10	1
23	Fipronil sulfide	120067-83-6	10	1
24	Heptachlor	76-44-8	50	5
25	Heptachlor epoxide (cis&trans) or (B+A)	1024-57-3 & 28044-83-9	50+50	5+5
26	Hexachlorobenzene (HCB)	118-74-1	50	5

Cmpd #	Pesticide	CAS #	Stock Conc. (µg/mL ethyl acetate)	Spiking Solution Conc. (µg/mL ethyl acetate)
27	Lindane (BHC gamma)	58-89-9	80	8
28	MGK-264 (isomers 1&2)	113-48-4	100	10
29	Metolachlor	51218-45-2	20	2
30	Nonachlor cis	5103-73-1	30	3
31	Nonachlor trans	39765-80-5	30	3
32	Oxychlordane	27304-13-8	20	2
33	Pentachloroaniline (PCA)	527-20-8	50	5
34	Pentachlorobenzene (PCB)	608-93-5	20	2
35	Permethrin (cis&trans)	52645-53-1	50	5
36	Pronamide	23950-58-5	10	1
37	Tefluthrin	79538-32-2	10	1

Table 8: Mixed Pesticides spiking solution in Ethyl Acetate

Solution	Procedure
Mixed Pesticide Spiking Solution	1) Measure 5 mL of each pesticide standard and add to a 50 mL volumetric flask. 2) Dilute to volume with ethyl acetate. 3) Mix well and transfer to glass storage container. 4) Store in freezer at < -10 °C. *Solution expires 1 year after preparation.*

Table 9: Preparation of Internal Standard

Solution	Procedure
Internal Standard Spiking Solution (20 µg/mL Trichloronate & 10 µg/mL Ethoprofos)	1) Measure 1.0 mL of the pesticide internal standard and add to a 50 mL volumetric flask. 2) Dilute to volume with ethyl acetate. 3) Mix well and transfer to glass storage container. 4) Store in freezer at < -10 °C. *Solution expires 1 year after preparation.*

Table 10: Preparation of Injection Standard for LC and GC compounds

Solution	Procedure
Muscle Injection Standard for LC compounds	1) Measure 200 µL of internal standard spiking solution and add to a 10 mL volumetric flask. 2) Measure 200 µL of mixed pesticide spiking solution and add to the same flask. 3) Dilute to volume with acetonitrile. 4) Mix well and transfer to glass storage container. 5) Store in freezer at < - 10 °C. *Solution expires 1 month after preparation.*
Egg Injection Standard for LC compounds	1) Measure 33.3 µL of internal standard spiking solution and add to a 10 mL volumetric flask. 2) Measure 33.3 µL of mixed pesticide spiking solution and add to the same the flask. 3) Dilute to volume with acetonitrile. 4) Mix well and transfer to glass storage container. 5) Store in freezer at < - 10 °C. *Solution expires 1 month after preparation.*
Muscle Injection Standard for GC compounds	1) Measure 200 µL of internal standard spiking solution and add to a 10 mL volumetric flask. 2) Measure 200 µL of mixed pesticide spiking solution and add to the same flask. 3) Dilute to volume with toluene. 4) Mix well and transfer to glass storage container. 5) Store in freezer at < -10 °C. *Solution expires 1 month after preparation.*
Egg Injection Standard for GC compounds	1) Measure 33.3 µL of internal standard spiking solution and add to 10 mL volumetric flask. 2) Measure 33.3 µL of mixed pesticide spiking solution and add to the same flask. 3) Dilute to volume with toluene. 4) Mix well and transfer to glass storage container. 5) Store in freezer at < -10 °C. *Solution expires 1 month after preparation.*

Table 11: Concentration of GC and LC Injection Standard

Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
	LC Mixed Standard		
1	3-Hydroxycarbofuran	0.02	0.00333
2	Acephate	0.04	0.00666
3	Acetamiprid	0.02	0.00333
4	Alachlor	0.02	0.00333
5	Aldicarb	0.04	0.00666
6	Aldicarb sulfone	0.04	0.00666
7	Aldicarb sulfoxide	0.1	0.0167
8	Atrazine	0.04	0.00666
9	Azinphos methyl	0.04	0.00666
10	Azoxystrobin	0.02	0.00333
11	Benoxacor	0.02	0.00333
12	Boscalid	0.06	0.00999
13	Buprofezin	0.1	0.0167
14	Carbaryl	0.1	0.0167
15	Carbofuran	0.02	0.00333
16	Carfentrazone ethyl	0.02	0.00333
17	Clothianidin	0.04	0.00666
18	Coumaphos O	0.04	0.00666
19	Coumaphos S	0.04	0.00666
20	Desethylatrazine	0.04	0.00666
21	Diazinon	0.02	0.00333
22	Dichlorvos (DDVP)	0.04	0.00666
23	Difenoconazole	0.06	0.00999
24	Diflubenzuron	0.05	0.00833
25	Dimethoate	0.04	0.00666
26	Diuron	0.32	0.0533
27	Ethion	0.04	0.00666
28	Ethofumesate	0.08	0.0133
29	Fenoxaprop ethyl	0.04	0.00666
30	Fluridone	0.1	0.0167

Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
31	Fluroxypyr-1-Methylheptyl-Ester	0.02	0.00333
32	Fluvalinate	0.03	0.005
33	Hexazinone	0.12	0.02
34	Hexythiazox	0.04	0.00666
35	Imazalil	0.02	0.00333
36	Imidacloprid	0.1	0.0167
37	Indoxacarb	0.1	0.0167
38	Linuron	0.1	0.0167
39	Malathion	0.16	0.0266
40	Metalaxyl	0.04	0.00666
41	Methamidophos	0.04	0.00666
42	Methomyl	0.12	0.02
43	Methoxyfenozide	0.02	0.00333
44	Metribuzin	0.2	0.0333
45	Myclobutanil	0.04	0.00666
46	Norflurazon	0.04	0.00666
47	Omethoate	0.04	0.00666
48	Piperonyl butoxide	0.09	0.015
49	Pirimiphos methyl	0.04	0.00666
50	Prallethrin	0.16	0.0266
51	Profenofos	0.04	0.00666
52	Propachlor	0.04	0.00666
53	Propanil	0.1	0.0167
54	Propetamphos	0.03	0.005
55	Propiconazole	0.06	0.00999
56	Pyraclostrobin	0.2	0.0333
57	Pyrethrin I	0.184	0.0306
58	Pyridaben	0.036	0.00599
59	Pyriproxyfen	0.08	0.0133
60	Resmethrin (cis&trans)	0.2	0.0333
61	Simazine	0.04	0.00666
62	Sulprofos	0.1	0.0167

Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
63	Tebufenozide	0.16	0.0266
64	Tetrachlorvinphos	0.04	0.00666
65	Tetraconazole	0.02	0.00333
66	Thiabendazole	0.06	0.00999
67	Thiamethoxam	0.04	0.00666
68	Thiobencarb	0.2	0.0333
69	Trifloxystrobin	0.02	0.00333
	GC Mixed Standard		
1	1-Naphthol	0.12	0.02
2	Aldrin	0.1	0.0167
3	Bifenthrin	0.02	0.00333
4	Chlordane cis	0.04	0.00666
5	Chlordane trans	0.04	0.00666
6	Chloroneb	0.036	0.00599
7	Chlorothalonil	0.24	0.04
8	Chlorpropham	0.12	0.02
9	Chlorpyrifos	0.03	0.005
10	Chlorpyrifos methyl	0.02	0.00333
11	DDD o,p'	0.2	0.0333
12	DDD p,p' + DDT o,p'	0.2 + 0.2	0.0333 + 0.0333
13	DDE o,p'	0.2	0.0333
14	DDE p,p'	0.2	0.0333
15	DDT p,p'	0.2	0.0333
16	Dieldrin	0.1	0.0167
17	Endosulfan I	0.2	0.0333
18	Endosulfan II	0.2	0.0333
19	Endosulfan sulfate	0.2	0.0333
20	Fenpropathrin	0.1	0.0167
21	Fipronil	0.02	0.00333
22	Fipronil desulfinyl	0.02	0.00333
23	Fipronil sulfide	0.02	0.00333
24	Heptachlor	0.1	0.0167

Cmpd #	Name	Muscle Injection Standard Conc. (µg of pest./mL of solution)	Egg Injection Standard Conc. (µg of pest./mL of solution)
25	Heptachlor epoxide (cis&trans) or (B+A)	0.1 + 0.1	0.0167 + 0.0167
26	Hexachlorobenzene (HCB)	0.1	0.0167
27	Lindane (BHC gamma)	0.16	0.0266
28	MGK-264 (isomers 1&2)	0.2	0.0333
29	Metolachlor	0.04	0.00666
30	Nonachlor cis	0.06	0.00999
31	Nonachlor trans	0.06	0.00999
32	Oxychlordane	0.04	0.00666
33	Pentachloroaniline (PCA)	0.1	0.0167
34	Pentachlorobenzene (PCB)	0.04	0.00666
35	Permethrin (cis&trans)	0.1	0.0167
36	Pronamide	0.02	0.00333
37	Tefluthrin	0.02	0.00333
Internal Standards			
	Ethoprophos (LC-MS/MS)	0.2	0.0333
	Trichloronate (GC-MS/MS)	0.4	0.0666

Sample Preparation

Samples must be kept cold before and during shipping to the laboratory. Once received at the laboratory, muscle samples must be frozen (≤ -10 °C) prior to grinding if they cannot be prepared on the day of receipt. Once frozen, temper (partially thaw) while keeping it as cold as possible. As shown in Figure 2, trim away fat and connective tissue. Grind tissue in blender or vertical cutter-mixer until homogeneous, as shown in Figure 3. Both liquid and powdered egg products require no sample preparation. An example of a liquid egg product is shown in Figure 4. Store samples frozen (≤ -10 °C) prior to analysis.

Figure 2: Prepared lean muscle sample with connective tissue removed. Photo courtesy of Hue Quach, USDA-FSIS.

Figure 3: Homogenized sample. Photo courtesy of Hue Quach, USDA-FSIS

Figure 4: Liquid egg samples. Photo courtesy of Hue Quach USDA-FSIS.

Pesticides Extraction

Samples

Weigh 20.0 ± 0.20 g of homogenized muscle sample, 5.0 ± 0.04 g of liquid egg product, or 2.5 ± 0.04 g powdered egg product into a 50 mL polypropylene centrifuge tube, as shown in Figure 5.

KEY DEFINITIONS

Negative control (Blank): A quality control sample that is negative for all analytes of interest.

Decision level control: Sample is prepared with addition of analytes that have a concentration level comparable to MLA. Negative and positive controls are compared to "Decision level control."

Recovery (positive control): Sample is prepared with addition of analytes that have a concentration level comparable to MLA. Samples are compared to recovery.

Figure 5: Weighed controls and samples. Photo courtesy of Ryan Matsuda, USDA-FSIS

QUALITY CONTROL

1. Weigh 3 portions of appropriate blank matrix into 50 mL polypropylene centrifuge tubes. One for the blank (negative control), one for the decision level control, and one for the positive control. Weigh one additional portion for a check sample, if applicable.
   a. For muscle tissue: 20.0 ± 0.2 g
   b. For liquid egg: 5.0 ± 0.04 g
   c. For powdered egg: 2.5 ± 0.04 g
2. Prepare decision level and recovery control by fortifying the sample with 100 µL for muscle or 25 µL for the liquid and powdered egg of the appropriate fortification standard.
3. Allow the sample to dry, about five minutes (min), before continuing to the extraction.

Extraction

1) Add 30 mL of ethyl acetate to each sample as shown in Figure 6.
2) Fortify all samples and controls with 100 µL, for muscle, or 25 µL, for eggs, of the internal standard spiking solution and cap centrifuge tube. Invert, vortex, or shake tubes to homogenize (or shred) tissue and ensure solvent reaches the entire sample.
3) Place samples on the shaker for one minute to mix.

Figure 6: Ethyl acetate added to controls and samples Photo courtesy of Ryan Matsuda USDA-FSIS

4) Add QuEChERS Salts Packets (8 g of $MgSO_4$ and 2 g NaCl) to each sample and cap tube as shown in Figure 7. Invert, vortex, or shake tubes to homogenize (or shred) tissue so the salt is evenly distributed and ensure solvent reaches the entire sample as shown in Figure 8.

Figure 7: Samples with QuEChERS salts added. Photo courtesy of Ryan Matsuda USDA-FSIS

Figure 8: Samples with QuEChERS salts. Photo courtesy of Jason Stone USDA-FSIS

Key Fact:

Make sure the solvent interacts well with the entire sample and the crystalline agglomerates are broken up sufficiently.

5) Shake vigorously for 5 min on the shaker.
6) Place samples into the ≤ -20 °C freezer for 30 min.
7) Remove samples from freezer and centrifuge at 3000 RCF for 8 min.
8) As shown in Figure 9, decant more than 18 mL of the ethyl acetate layer into a 50 mL graduated glass centrifuge tube using a funnel and filter paper.

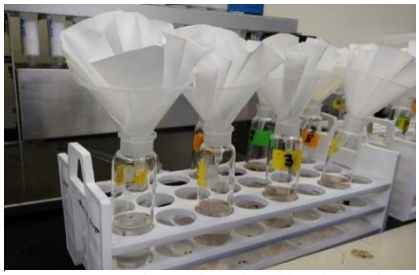

Figure 9: Samples undergoing filtration. Photo courtesy of Ryan Matsuda USDA-FSIS

9) Adjust the volume of muscle samples to 18 mL and of egg samples to 12 mL, discarding the excess.
10) Concentrate the extract under nitrogen in a 65 ± 5 °C water bath until the volume remains constant. This volume is typically 0.5 mL to 2.0 mL, as shown in Figure 10.
11) Dilute to 15 mL with acetonitrile, cap glass tube and vortex for one minute.
12) Place samples in ≤ -70 °C freezer for 30 min.
13) After removing samples from the freezer, briefly let samples rest on a rack until stopper is able to loosen. This will prevent pressure from building up while in the centrifuge and tubes breaking.
14) Centrifuge the extract while frozen for 3.5 min at 1050 RCF.

Figure 10: Samples undergoing evaporation. Photo courtesy of Ryan Matsuda USDA-FSIS

Key Fact:

Acetonitrile will thaw during centrifugation.

15) After centrifugation, to minimize disruption of the pellet, transfer 10 mL to another vessel prior to SPE.

16) Prepare a solid phase extraction (SPE) column by adding, approximately 2 g of anhydrous $MgSO_4$ to the top of the 1000 mg C_{18} SPE Columns.

Key Fact:

To save time during the analysis, prepare SPE columns containing $MgSO_4$ ahead of time and store in a desiccator.

17) Using a positive pressure SPE manifold, condition the SPE cartridge with 5 mL of 1% acetic acid/acetonitrile, and elute to waste.

18) Place labeled 15 mL graduated glass tubes in the collection rack below SPE cartridges, as shown in Figure 11.

19) Transfer the 10 mL of sample extract into the SPE column from the previous vessel with a pipette. During transfer, ensure that SPE columns are not overfilled. Elute the extract through the column using a regulated flow pressure of not greater than 35 psi.

Figure 11: Solid phase extraction manifold. Photo courtesy of Ryan Matsuda USDA-FSIS

   a. Due to limited volume space in SPE column, this step will need to be repeated several times until all 10 mL are passed through the SPE column. Do not allow the SPE column to go dry.

20) After the extract has completely passed through the column, add two aliquots of 2.5 mL of 1% acetic acid/acetonitrile to elute the sample from the column.

21) Set manifold to full flow to fully elute and dry the column for 1 min.

Optional Stopping Point:

This is an optional stopping point. If stopping overnight, samples should be capped and stored at ≤ -20 °C. The extracts are stable for 24 hours when stored at ≤ -20 °C.

22) As shown in Figure 12, concentrate each sample to less than 2 mL (final sample volume) under nitrogen in a 65 ± 5 °C water bath. Adjust all samples to 2 mL with acetonitrile.

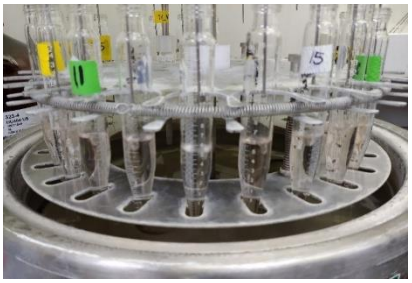

Figure 12: Samples undergoing evaporation. Photo courtesy of Ryan Matsuda USDA-FSIS

Prepare Extract for UHPLC-MS/MS Analysis

1) Transfer 1 mL of the extract from step 22 to a 2 mL micro-centrifuge tubes with QuEChERS salts (150 mg $MgSO_4$ & 50 mg Primary Secondary Amine (PSA)).
2) Vortex the micro-centrifuge tubes for 1 min.
3) As shown in Figure 13, centrifuge the mini-centrifuge tubes for 2 min at 10,000 RCF.
4) Transfer the sample extract to a 3 mL plastic syringe with a 0.2 µm Nylon syringe filter and filter extract into a labeled autosampler vial. Analyze vial by UHPLC-MS/MS.

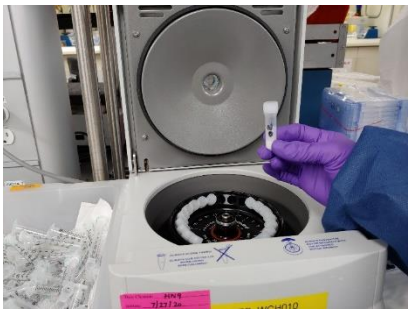

Figure 13: Samples undergoing centrifugation. Photo courtesy of Ryan Matsuda USDA-FSIS

Prepare Extract for GC-MS/MS Analysis

1) Using a positive pressure SPE manifold , condition a 500 mg PSA (Primary Secondary Amine) SPE column with 4 mL of 3:1 v/v acetone/toluene and elute to waste.
2) Place labeled 15 mL graduated glass tubes in the collection rack below SPE columns, as illustrated in Figure 14.
3) Transfer the remainder of the sample extract with from step 22 to the SPE column with a pipette.
4) Elute the extract through the column using a regulated flow pressure of not greater than 35 psi with 4 mL of 3:1 v/v acetone/toluene.

Figure 14: Samples undergoing SPE cleanup for GC-MS/MS Analysis. Photo courtesy of Ryan Matsuda USDA-FSIS

5) Collect the eluate while washing the SPE column two times with 4 mL of 3:1 v/v acetone/toluene (eluant). Do not allow the SPE column to go dry.
6) After the last 4 mL portion of eluant has passed through the column, set the manifold to full flow to fully elute and dry the column for one minute .
7) Evaporate the sample to less than 0.5 mL under nitrogen in a 65 ± 5 °C water bath.
8) Add 3 mL of toluene to graduated glass tube and vortex.
9) Evaporate again to less than 0.5 mL to ensure all other solvents have been removed.
10) Bring the volume to 1.0 mL with toluene and vortex to mix.
11) Transfer the sample to a labeled autosampler vial. Analyze by GC-MS/MS.

Instrumental Analysis

An example of a UHPLC-MS/MS instrument and a GC-MS/MS instrument are shown in Figure 15 and Figure 16, respectively.

UHPLC-MS/MS Instrumental Settings

Chromatographic Parameters

1) Mobile phases for pesticide analysis
    a. LC/MS/MS Mobile Phase A (5 mM ammonium acetate in 0.1% formic acid in water (v/v))
    b. LC/MS/MS Mobile Phase B (0.1% formic acid in methanol (v/v))
2) Flow rate: 0.5 mL/min
3) Run time: 12 min
4) Gradient Program

Table 13: UHPLC Gradient Program

Time (min)	% Mobile Phase A	% Mobile Phase B	Gradient
Initial	90%	10%	None
0.25	90%	10%	None
7.75	2%	98%	Linear
10.50	2%	98%	Linear
10.55	90%	10%	None
12	90%	10%	Linear

5) Autosampler program
    a. Run time: 12 min
    b. Injection needle: 15 µL
    c. Sample injection mode: Flow through needle
    d. Injection volume: 1 µL for muscle samples, 2 µL for egg samples
    e. Weak Wash: 10% methanol in water
    f. Strong Wash: 0.5% formic acid in 1:1:1:1 acetonitrile: methanol: isopropanol: water
6) Column manager
    a. Column valve position: To match column location
    b. Column manager temperature: 50 °C

Mass Spectrometry Parameters

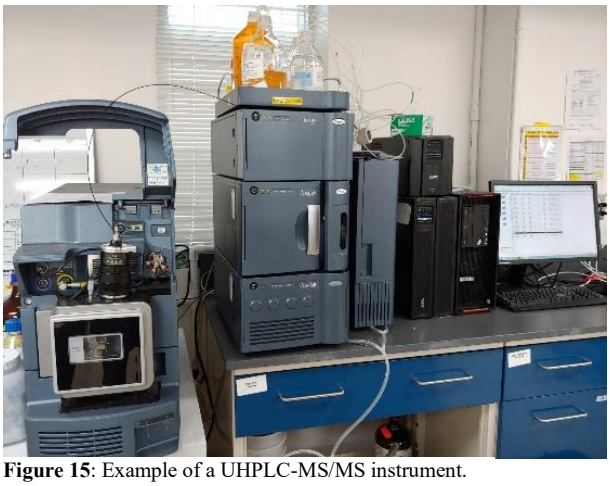

Figure 15: Example of a UHPLC-MS/MS instrument.
Photo courtesy of Ryan Matsuda USDA-FSIS

1) Type: MS/MS
2) Electrospray Source Parameters
   a. Capillary (kV): 3.5
   b. Multiplier: - 640V
   c. Dwell time: varied from 0.025-0.2s
   d. Cone (V): Variable - analyte dependent
   e. Extractor (V): 3.0
   f. RF (V): 0.10
   g. Source Temperature (°C): 150
   h. Desolvation Temperature (°C): 350
   i. Cone Gas Flow (L/hr): 25
   j. Desolvation Gas Flow (L/hr): 650
   k. Collision Gas Flow (mL/min): 0.25
3) Analyzer Parameters
   a. LM1 Resolution 3.5
   b. HM 1 Resolution: 15
   c. Ion Energy 1: -0.8
   d. MSMS Mode Entrance: -5
   e. MSMS Mode Collision Energy: Variable – analyte dependent
   f. MSMS Mode Exit: 1
   g. LM 2 Resolution: 12.50
   h. HM 2 Resolution: 12.50
   i. Ion Energy 2: 0.2
4) MS Method Parameters:
   a. Type: MRM
   b. Ion Mode: ES+
   c. MRM Transitions:

Table 14: LC MRM Transitions

Cmpd #	Pesticide	RT (min)	Cone (V)	First transition (m/z)	Coll En (V)	Second transition (m/z)	Coll En (V)	Quant Ion
1	3-Hydroxycarbofuran	3.57	15	255.2 < 163	18	255.2 < 181	15	163
2	Acephate	1.53	20	184.1 < 125	16	184.1 < 143	12	143

Cmpd #	Pesticide	RT (min)	Cone (V)	First transition (m/z)	Coll En (V)	Second transition (m/z)	Coll En (V)	Quant Ion
3	Acetamiprid	3.57	40	223 < 56	16	223 < 126	16	126
4	Alachlor	6.61	27	269.8 < 161.9	19	269.8 < 237.8	11	237.8
5	Aldicarb	4.23	12	190.8 < 88.7	13	190.8 < 115.8	5	115.8
6	Aldicarb sulfone	2.16	23	223 < 76	7	223 < 86	12	86
7	Aldicarb sulfoxide	1.98	16	207 < 89	14	207 < 132	10	89
8	Atrazine	5.49	35	216.1 < 104	26	216.1 < 174.1	18	174.1
9	Azinphos methyl	5.82	22	317.7 < 124.8	35	317.7 < 131.9	30	131.9
10	Azoxystrobin	6.01	30	404.1 < 344.2	26	404.1 < 372.1	14	372.1
11	Benoxacor	5.86	22	259.7 < 133.8	29	259.7 < 148.9	17	148.9
12	Boscalid	6.17	22	342.8 < 271.3	33	342.8 < 306.7	19	306.7
13	Buprofezin	7.52	22	306 < 115.9	15	306 < 201	11	201
14	Carbaryl	5.04	20	202.2 < 127	28	202.2 < 145	15	145
15	Carbofuran	4.87	25	222.2 < 123	23	222.2 < 165	13	123
16	Carfentrazone ethyl	6.83	37	412 < 345.7	23	412 < 365.6	17	345.7
17	Clothianidin	3.23	25	250.1 < 132.1	29	250.1 < 168.6	15	168.6
18	Coumaphos O	5.91	45	347 < 211	34	347 < 291	22	291
19	Coumaphos S	6.95	40	363 < 227	24	363 < 307	16	227
20	Desethylatrazine	3.89	35	187.9 < 104	28	187.9 < 146	20	146
21	Diazinon	6.97	36	305.1 < 153.1	22	305.1 < 169.1	18	169.1
22	Dichlorvos	4.7	32	220.7 < 108.8	19	220.7 < 144.8	11	108.8
23	Difenoconazole	7.16	42	406 < 250.8	25	406 < 336.8	17	250.8
24	Diflubenzuron	6.65	23	311 < 141.1	32	311 < 158.2	15	158.2
25	Dimethoate	3.5	17	230 < 125	20	230 < 199	10	199
26	Diuron	5.6	25	233 < 72.1	15	233 < 160	28	72.1
27	Ethion	7.6	22	384.7 < 142.8	25	384.7 < 198.8	11	198.8
28	Ethofumesate	6.01	13	304.1 < 121.1	20	304.1 < 161.2	25	121.1
ISTD	Ethoprofos	6.57	23	243.1 < 173	22			173
29	Fenoxaprop ethyl	7.43	12	361.9 < 243.7	25	361.9 < 287.7	19	287.7
30	Fluridone	5.9	22	330 < 258.9	45	330 < 309.2	33	309.2
31	Fluroxypyr-1methylheptyl-ester	7.76	20	367 < 209	22	367 < 255	10	255
32	Fluvalinate	8.14	27	502.8 < 180.7	27	502.8 < 207.8	13	207.8
33	Hexazinone	4.89	32	252.9 < 70.9	33	252.9 < 170.8	17	170.8
34	Hexythiazox	7.72	30	353 < 168.1	26	353 < 228.1	14	228.1
35	Imazalil	5.24	30	297 < 159	36	297 < 255	20	159
36	Imidacloprid	3.2	25	256.1 < 175	18	256.1 < 209	14	209
37	Indoxacarb	7.2	25	528 < 150.1	22	528 < 203.2	35	150.1
38	Linuron	5.99	28	249 < 160	18	249 < 182	17	160
39	Malathion	6.26	22	330.7 < 126.8	11	330.7 < 284.7	7	126.8
40	Metalaxyl	5.65	18	280.1 < 192.2	18	280.1 < 220.1	13	220.1
41	Methamidophos	1.21	22	142 < 94	14	142 < 125	13	94
42	Methomyl	2.51	13	163.1 < 88	9	163.1 < 106	9	88
43	Methoxyfenozide	6.32	15	369.1 < 91.1	47	369.1 < 149.2	18	149.2
44	Metribuzin	4.8	32	214.8 < 83.8	21	214.8 < 186.7	19	186.7

Cmpd #	Pesticide	RT (min)	Cone (V)	First transition (m/z)	Coll En (V)	Second transition (m/z)	Coll En (V)	Quant Ion
45	Myclobutanil	6.32	28	289.1 < 70.1	18	289.1 < 125.1	30	70.1
46	Norflurazon	5.66	30	304.1 < 160.1	40	304.1 < 284.1	32	284.1
47	Omethoate	1.79	20	214 < 155	14	214 < 183	12	183
48	Piperonyl butoxide	7.63	12	356 < 118.9	37	356 < 176.8	13	176.8
49	Pirimiphos methyl	7.08	12	305.9 < 107.8	33	305.9 < 163.9	21	107.8
50	Prallethrin	7.22	22	301.1 < 132.9	11	301.1 < 168.9	9	132.9
51	Profenofos	7.43	35	374.8 < 304.9	18	374.8 < 346.8	14	304.9
52	Propachlor	5.58	17	211.8 < 105.8	25	211.8 < 169.7	17	169.7
53	Propanil	6.01	32	217.8 < 126.8	27	217.8 < 161.7	15	161.7
54	Propetamphos	6.34	17	281.9 < 137.8	19	281.9 < 155.7	11	137.8
55	Propiconazole	6.95	27	341.8 < 68.9	21	341.8 < 158.8	27	68.9
56	Pyraclostrobin	7.01	40	387.8 < 163.7	12	387.8 < 194.1	10	194.1
57	Pyrethrin I	7.87	22	329.2 < 143	15	329.2 < 161	9	161
58	Pyridaben	8.09	25	365.2 < 147.1	28	365.2 < 309	13	147.1
59	Pyriproxyfen	7.62	12	322 < 95.8	15	322 < 184.8	23	95.8
60	Resmethrin	8.15	12	356.2 < 127.9	41	356.2 < 170.8	15	170.8
61	Simazine	4.85	35	202 < 124.1	20	202 < 132	20	132
62	Sulprofos	7.72	27	322.9 < 218.7	17	322.9 < 246.8	13	218.7
63	Tebufenozide	6.73	12	353.1 < 105	50	353.1 < 133.1	22	133.1
64	Tetrachlorvinphos	6.77	27	366.5 < 126.7	17	366.5 < 240.6	17	126.7
65	Tetraconazole	6.5	37	371.9 < 69.8	23	371.9 < 158.7	33	158.7
66	Thiabendazole	3.25	45	202.1 < 131	33	202.1 < 175	24	175
67	Thiamethoxam	2.63	23	292 < 181	18	292 < 211	13	211
68	Thiobencarb	7.1	25	257.9 < 100.1	10	257.9 < 125.1	20	125.1
69	Trifloxystrobin	7.24	25	409 < 145	44	409 < 186	20	186

GC-MS/MS Instrumental Settings

GC Chromatographic Parameters

Figure 16: Example of a GC-MS/MS instrument. Photo courtesy of Ryan Matsuda USDA-FSIS

1) Carrier Gas: Helium
2) Column 1 Flow Rate: 1.4 mL/min
3) Column 2 Flow Rate: 1.2 mL/min
4) Injector temperature: 280 ºC
5) Injection volume: 1 µL for muscle samples, 2 µL for egg
6) Injection Mode: splitless
7) Temperature program
   a. Initial temp: 60 ºC
   b. Initial hold time: 1 min
   c. Program rate up to 120 ºC: 40 ºC/min
   d. Program rate up to 292: 5 ºC/min
   e. Post-run time: 2 min
   f. Total Run time: 36.9 min
   g. Solvent delay: 9 min

GC Mass Spectrometry Parameters

1) Type: MS/MS
2) Instrument Parameters:
   a. Ionization: Positive Electron Impact
   b. Detector EMV: 1352 V
   c. Collision Gas: Nitrogen @ 1.5 mL/Min
   d. Collision Energy: Variable – analyte dependent
   e. MS Source temperature: 300 ºC
   f. Transfer line temperature: 300 ºC
   g. Solvent delay: 7.0 min
   h. Autotune the instrument as needed.
   i. MRM Transitions:

Table 15: GC MRM Transitions

Cmpd #	Pesticide	RT (min)	First transition (m/z)	Coll En (V)	Second transition (m/z)	Coll En (V)	Third transition (m/z)	Coll En (V)	Quant Ion
1	1-Naphthol	9.479	144 > 115	25	115 > 89	20			115
2	Aldrin	19.54	263 > 193	55	263 > 228	35	263 > 191	55	193
3	Bifenthrin	31.76	181 > 165	10	181 > 166	20	165 > 115	40	165

Cmpd #	Pesticide	RT (min)	First transition (m/z)	Coll En (V)	Second transition (m/z)	Coll En (V)	Third transition (m/z)	Coll En (V)	Quant Ion
4	Chlordane cis	23.77	373 > 266	25	373 > 337	20	373 > 264	25	266
5	Chlordane trans	23.08	373 > 265.9	15	373 > 337	10	373 > 264	20	265.9
6	Chloroneb	9.101	191 > 113	15	191 > 141	10			113
7	Chlorothalonil	15.34	266 > 132.9	30	266 > 168	60	266 > 231	20	132.9
8	Chlorpropham	11.88	213 > 127	20	213 > 171	5			127
9	Chlorpyrifos	19.57	316 > 260	15	314 > 166	40	314 > 286	5	260
10	Chlorpyrifos methyl	17.16	286 > 93	35	286 > 271	35	286 > 208	25	93
11	DDD o,p'	25.57	237 > 165	20	235 > 199	10	199 > 164	20	165
12	DDD p,p' + DDT o,p'	27.66	235 > 165	20	199 > 164	20	235 > 199	15	165
13	DDE o,p'	23.32	246 > 176	30	318 > 248	15	318 > 246	15	176
14	DDE p,p'	25.12	246 > 176	30	318 > 248	15	318 > 246	15	176
15	DDT p,p'	29.71	235 > 165	20	235 > 199	15	199 > 164	15	165
16	Dieldrin	25.32	277 > 241	5	263 > 193	60	272 > 237	10	241
17	Endosulfan I	23.75	241 > 205.9	20	339 > 160	20			205.9
18	Endosulfan II	27.32	241 > 206	20	339 > 160	20			206
19	Endosulfan sulfate	29.58	272 > 237	15	272 > 235	30	272 > 143	30	237
20	Fenpropathrin	32.1	181 > 152	25	265 > 210	10			152
21	Fipronil	22.12	367 > 213	60	367 > 255	35			213
22	Fipronil desulfinyl	17.74	388 > 333	20	333 > 231	60			333
23	Fipronil sulfide	21.59	351 > 255	20	420 > 351	10			255
24	Heptachlor	17.7	272 > 237	15	337 > 266	15			237
25	Heptachlor epoxide (cis&trans) or (B+A)	22.04	183 > 119	25	272 > 237	20	353 > 282	25	119
26	Hexachlorobenzene (HCB)	12.92	284 > 249	15	282>247	60	250 > 142	45	249
27	Lindane (BHC gamma)	14.36	181 > 145	15	219 > 183	5	219 > 109	35	145
28	MGK – 264 1	20.8	164 > 98	10	164 > 67	5	164 > 80	35	98
	MGK – 264 2	21.54	164 > 67	15	164 > 98	10	164 > 80	35	67
29	Metolachlor	19.46	238 > 162	10	162 > 133	15			162
30	Nonachlor cis	27.46	409 > 108.9	15	409 > 302	20			108.9
31	Nonachlor trans	23.9	409 > 302	25	409 > 109	40	409 > 263	40	302
32	Oxychlordane	21.69	187 > 123	10	187 > 85	30			123
33	Pentachloroaniline (PCA)	16.54	265 > 192	25	265 > 228	35			192
34	Pentachlorobenzene (PCB)	9.348	250 > 142	35	250 > 179	30			142
35	Permethrin (cis&trans)	34.21	183 > 153	15	183 > 165	10	183 > 127	45	153
36	Pronamide	14.71	173 > 145	15	173 > 109	55			145
37	Tefluthrin	15.35	177 > 127	15	177 > 137	20	177 > 87	60	127
ISTD	Trichloronate	20.38	297 > 269	10	299 > 271	10			269

Instrument Note

All chromatographic and instrument parameters were optimized in accordance with FSIS laboratory system method performance requirements and during annual preventative maintenance and calibration.

- Retention time windows, collision energies, and selected masses for precursor and product ions were set and utilized at time of method validation.
  - Retention time windows may be adjusted to account for aging of UHPLC columns, GC columns, or for improved separation to ensure that all chromatographic peaks are present.
  - Collision energies may be adjusted and optimized for improved mass spectrometry detection.
  - Target masses for precursor and product ions can be optimized to a *m/z* value that falls within the unit mass resolution of the exact mass, but not to exceed the next integer value (e.g., if the exact mass is 787.5, an allowable target mass range includes 787.0-787.9).
- Parameter modifications to improve instrument performance to ensure all chromatographic peaks are present must meet the acceptance criteria listed in the method's Quality Assurance Plan.
- Significant changes that affect method performance require equivalency testing and FSIS laboratory leadership approval.

Sample Set

The injection sequence below can be modified, as needed, but must include required controls. System Suitability is to be demonstrated prior to sample set injection.

1) Injection Standard
2) DL Control
3) Positive Control (Recovery)
4) Solvent Blank
5) Negative Control (Blank)
6) Intra-Laboratory Check Sample (if applicable)
7) Samples, up to a maximum of 18
8) Re-injection of the positive control (recovery) (for system suitability)

INTRA-LABORATORY

CHECK SAMPLE

Defined on the CLG website.

Reporting of Results

Decision Criteria

Screening

1) All ions listed in table 14 and 15 for the analyte must be present.
2) All ions must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection.
3) The internal standard response for the sample must be > 50% of the internal standard response of the recovery (positive control). If the internal standard response of the sample exceeds 200% of the internal standard response of the recovery (positive control), that sample will be investigated.
4) Retention time for the recovery and samples must match the retention time of the decision level recovery within ± 5% for LC, ± 0.5% for 1-naphthol and chloroneb, ± 1% for all other single peak GC compounds, and ± 5% for multipeak compounds for GC.
5) All quantitative ion peak areas in the blank must be < 10% of the decision level recovery.
6) The sample is screen positive if the following criteria are met:
    a. The fortified recovery of the analyte must exceed 10% of the decision level recovery.
    b. The sample's relative response factor equals or exceeds the recovery response factor.

QUALITY CONTROL

Quality Control Procedures

1. For set acceptance, 95% (for LC and for GC) of the monitored analytes in the recovery (positive control) must meet screening criteria. For sample reporting purposes, screen positive analytes must meet screening criteria in the recovery (positive control), or else further testing is warranted.
2. For set acceptance, 95% (for LC and for GC) of the monitored analytes in the blank (negative control) must not meet the screening criteria. The blank (negative control) must be negative using the criteria in screening criteria for samples containing corresponding presumptive positive analytes.
3. The internal standard response for the recovery (positive control) and blank (negative control) must be 50-150% of the internal standard response of the decision level.

Intra-laboratory Check Samples (If applicable)

1. Acceptability criteria.
    a. 95% of the monitored analytes in a fortified Intra-Laboratory Check must meet screening criteria.
    b. 95% of the monitored analytes in an unfortified Intra-Laboratory Check must be negative using the screening criteria.
    c. FSIS Field Service Laboratories are to refer to internal FSIS Quality Control Procedures when unacceptable values are obtained:
        i. Refer to LW-Q1002, Chemistry Non-Conformance Tables, for how to proceed and whether to take corrections or corrective actions.

Calculations

1) Relative Response Factor (RRF)

   This is the internal standard corrected analyte response.

   A = B / C

   where

   A = Relative Response Factor (unitless)

   B = Quant Ion Peak Area of Analyte (counts)

   C = Quant Ion Peak Area of Internal Standard (counts)

2) Estimated Amount Found

   This is a quantitative estimate calculated for comparison to the MLA. It is based on a one-point calibration with the recovery (positive control) as the reference. Program the MS instruments to automatically calculate this.

   D = E * A sample / A pos. ctrl.

   where

   D = Estimated Amount Found in the Sample (ppb)

   E = Recovery (positive control) Fortification Level (ppb)

   A sample = Relative Response Factor in the Sample (unitless)

   A pos. ctrl. = Relative Response Factor in the recovery (positive control) (unitless)

Minimum Level of Applicability

Table 16: Minimum Level of Applicability for Screening Level per species

#	Pesticides	Porcine (ppb)	Bovine (ppb)	Poultry (ppb)	Ovine (ppb)	Caprine (ppb)	Equine (ppb)	Catfish (ppb)	Liquid Eggs (ppb)	Powder Eggs (ppb)
LC Compounds										
Carbamates										
1	3-Hydroxycarbofuran	5	5	5	5	5	5	5	5	10
2	Aldicarb	10	10	10	10	10	10	10	N/App	N/App
3	Aldicarb sulfone	10	10	10	10	10	10	10	10	20
4	Aldicarb sulfoxide	25	25	25	25	25	25	25	25	50
5	Carbaryl	25	25	25	25	25	25	25	25	50
6	Carbofuran	5	5	5	5	5	5	5	5	10
7	Methomyl	30	30	30	30	30	30	30	30	60
8	Thiobencarb	50	50	50	50	50	50	50	50	100
Conazole / Triazole										
9	Difenoconazole	15	15	15	15	15	15	15	15	30
10	Myclobutanil	10	10	10	10	10	10	10	10	20
11	Propiconazole	15	15	15	15	15	15	15	15	30
12	Tetraconazole	5	5	5	5	5	5	5	5	10
Halogenated Pesticides										
13	Alachlor	5	5	5	5	5	5	5	5	10
14	Boscalid	15	15	15	15	15	15	15	15	30
15	Carfentrazone ethyl	5	5	5	5	5	5	5	5	10
16	Diflubenzuron	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25
17	Linuron	25	25	25	25	25	25	25	25	50
18	Norflurazon	10	10	10	10	10	10	10	10	20
19	Propachlor	10	10	10	10	10	10	10	10	20
20	Propanil	25	25	25	25	25	25	25	25	50
Neonicotinoids										
21	Acetamiprid	5	5	5	5	5	5	5	5	10
22	Clothianidin	10	10	10	10	10	10	10	10	20
23	Imidacloprid	25	25	25	25	25	25	25	25	50
24	Thiamethoxam	10	10	10	10	10	10	10	10	20
Organophosphates										
25	Acephate	10	10	10	10	10	10	10	N/App	N/App
26	Azinphos methyl	10	10	10	10	10	10	10	10	20
27	Coumaphos O	10	10	10	10	10	10	10	10	20
28	Coumaphos S	10	10	10	10	10	10	10	10	20
29	Diazinon	5	5	5	5	5	5	5	5	10
30	Dichlorvos (DDVP)	10	10	10	10	10	10	10	10	20
31	Dimethoate	10	10	10	10	10	10	10	10	20

#	Pesticides	Porcine (ppb)	Bovine (ppb)	Poultry (ppb)	Ovine (ppb)	Caprine (ppb)	Equine (ppb)	Catfish (ppb)	Liquid Eggs (ppb)	Powder Eggs (ppb)
32	Ethion	10	10	10	10	10	10	10	10	20
33	Malathion	40	40	40	40	40	40	40	40	80
34	Methamidophos	10	10	10	10	10	10	10	N/App	N/App
35	Omethoate	10	10	10	10	10	10	10	N/App	N/App
36	Pirimiphos methyl	10	10	10	10	10	10	10	10	20
37	Profenofos	10	10	10	10	10	10	10	10	20
38	Propetamphos	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	15
39	Sulprofos	25	25	25	25	25	25	25	25	50
40	Tetrachlorvinphos	10	10	10	10	10	10	10	10	20
	General Pesticides									
41	Azoxystrobin	5	5	5	5	5	5	5	5	10
42	Benoxacor	5	5	5	5	5	5	5	5	10
43	Buprofezin	25	25	25	25	25	25	25	25	50
44	Diuron	80	80	80	80	80	80	80	80	160
45	Ethofumesate	20	20	20	20	20	20	20	20	40
46	Fenoxaprop ethyl	10	10	10	10	10	10	10	10	20
47	Fluridone	25	25	25	25	25	25	25	25	50
48	Fluroxypyr-1-Methylheptyl-Ester	5	5	5	5	5	5	5	5	10
49	Hexazinone	30	30	30	30	30	30	30	30	60
50	Hexythiazox	10	10	10	10	10	10	10	10	20
51	Imazalil	5	5	5	5	5	5	5	N/App	N/App
52	Indoxacarb	25	25	25	25	25	25	25	25	50
53	Metalaxyl	10	10	10	10	10	10	10	10	20
54	Methoxyfenozide	5	5	5	5	5	5	5	5	10
55	Metribuzin	50	50	50	50	50	50	50	50	100
56	Piperonyl butoxide	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	45
57	Pyraclostrobin	50	50	50	50	50	50	50	50	100
58	Pyridaben	9	9	9	9	9	9	9	9	18
59	Pyriproxyfen	20	20	20	20	20	20	20	20	40
60	Tebufenozide	40	40	40	40	40	40	40	40	80
61	Thiabendazole	15	15	15	15	15	15	15	15	30
62	Trifloxystrobin	5	5	5	5	5	5	5	5	10
	Pyrethroids									
63	Fluvalinate	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	15
64	Prallethrin	40	40	40	40	40	40	40	40	80
65	Pyrethrin I	28	28	28	28	28	28	28	28	56
66	Resmethrin (cis&trans)	50	50	50	50	50	50	50	50	100
	Triazine									
67	Atrazine	10	10	10	10	10	10	10	N/App	N/App
68	Desethylatrazine	10	10	10	10	10	10	10	10	20

#	Pesticides	Porcine (ppb)	Bovine (ppb)	Poultry (ppb)	Ovine (ppb)	Caprine (ppb)	Equine (ppb)	Catfish (ppb)	Liquid Eggs (ppb)	Powder Eggs (ppb)
69	Simazine	10	10	10	10	10	10	10	10	20
	GC Compounds									
	Carbamate									
1	Chlorpropham	30	30	30	30	30	30	30	30	60
	Halogenated									
2	Pronamide	5	5	5	5	5	5	5	5	10
	Organochlorine									
3	Aldrin	25	25	25	25	25	25	25	25	50
4	Chlordane cis	10	10	10	10	10	10	10	10	20
5	Chlordane trans	10	10	10	10	10	10	10	10	20
6	DDD o,p’	50	50	50	50	50	50	50	50	100
7	DDD p,p’ + DDT, o,p'	50 +50	50+50	50+50	50+50	50+50	50+50	50+50	50+50	100+100
8	DDE o,p’	50	50	50	50	50	50	50	50	100
9	DDE p,p’	50	50	50	50	50	50	50	50	100
10	DDT p,p’	50	50	50	50	50	50	50	50	100
11	Dieldrin	25	25	25	25	25	25	25	25	50
12	Endosulfan I	50	50	50	50	50	50	50	50	100
13	Endosulfan II	50	50	50	50	50	50	50	50	100
14	Endosulfan sulfate	50	50	50	50	50	50	50	50	100
15	Heptachlor	25	25	25	25	25	25	25	25	50
16	Heptachlor epoxide (cis+ trans) or (B+A)	25+25	25+25	25+25	25+25	25+25	25+25	25+25	25+25	50+50
17	Hexachlorobenzene (HCB)	25	25	25	25	25	25	25	N/App	N/App
18	Lindane (BHC gamma)	40	40	40	40	40	40	40	40	80
19	Nonachlor cis	15	15	15	15	15	15	15	15	30
20	Nonachlor trans	15	15	15	15	15	15	15	15	30
21	Oxychlordane	10	10	10	10	10	10	10	10	20
22	Pentachlorobenzene (PCB)	10	10	10	10	10	10	10	10	20
	Organophosphate									
23	Chlorpyrifos	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	15
24	Chlorpyrifos methyl	5	5	5	5	5	5	5	5	10
	General Pesticides									
25	1-Naphthol	30	30	30	30	30	30	30	N/App	N/App
26	Fipronil	5	5	5	5	5	5	5	5	10
27	Fipronil desulfinyl	5	5	5	5	5	5	5	5	10
28	Fipronil sulfide	5	5	5	5	5	5	5	5	10
29	Metolachlor	10	10	10	10	10	10	10	10	20
30	MGK-264 (isomers 1 & 2)	50	50	50	50	50	50	50	50	100

Pyrethroids										
31	Bifenthrin	5	5	5	N/App	5	5	N/App	5	10
32	Fenpropathrin	25	25	25	25	25	25	25	25	50
33	Permethrin (cis&trans)	25	25	25	25	25	25	N/App	N/App	N/App
34	Tefluthrin	5	5	5	5	5	5	5	5	10
Substituted Benzenes										
35	Chloroneb	9	9	9	9	9	9	9	9	18
36	Chlorothalonil	60	60	60	60	60	60	60	N/App	120
37	Pentachloroaniline (PCA)	25	25	25	25	25	25	25	25	50

Safety Hazards

Table 17: Safety Hazards and Recommended Safe Procedures

Procedure Step	Hazard	Recommended Safe Procedures
Acetone, Acetonitrile, Ethyl Acetate, Isopropanol, Methanol, Toluene	Flammable	Keep in well-closed containers away from ignition sources. Avoid contact or prolonged exposure to vapors. Work in fume hood. Keep away from flame or heat.
Formic acid, Acetic acid	Corrosive, Caustic	Wear personal protective equipment, avoid skin contact.
Pesticide Standards	Some individuals may have allergic reactions to pesticide, which may cause skin and respiratory irritation. Possible reproductive toxicity.	Wear personal protective equipment, avoid skin contact. Handle with extreme caution. Work in a well-ventilated area.

References

1) The Environmental Protection Agency (EPA) regulates the approval and use of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act.

2) 40 CFR 180 for Tolerance values set by EPA.

Contact Information and Inquiries

Inquiries about methods can be submitted through the USDA website via the "Ask USDA" portal at https://ask.usda.gov or please contact:

Chemistry Section
Laboratory Quality Assurance, Response, and Coordination Staff
USDA/FSIS/OPHS
950 College Station Road
Athens, GA 30605
OPHS.LQAD@usda.gov

This method has been validated, reviewed, approved, and deemed suitable and fit for purpose for use in the USDA FSIS Field Service Laboratories.

William K. Shaw, Jr., PhD
Executive Associate for Laboratory Services