# USDA Pesticide Data Program Analytical Methods

## California Department of Food and Agriculture Multi-Residue Method

Three PDP laboratories (California, Texas, and Washington) use modified versions of the California Department of Food and Agriculture (CDFA) multi-residue method[1] (MRM) to extract fruit and vegetable samples for pesticide screening. Fifty grams of homogenized sample is extracted with acetonitrile. Sodium chloride is added to the sample/acetonitrile mixture and agitated. All samples are subjected to an initial C-18 solid phase extraction (SPE) clean-up step. The acetonitrile is evaporated from one aliquot of each sample, which is reconstituted with acetone for organophosphate analysis. Additional aliquots are cleaned up using florisil or aminopropyl SPE columns depending on the type of compound or the instrumental analysis to be conducted. Instrument/detector combinations used include gas chromatography (GC)/electron capture detector (ECD), GC/electrolytic conductivity detector (ELCD), GC/flame photometric detector (FPD), GC/halogen specific detector (XSD), GC/mass selective detector (MSD), high performance liquid chromatography (HPLC) with post- and/or pre-column derivatization and fluorescence detector (FD), liquid chromatography (LC)/mass spectrometer (MS), and LC/tandem mass spectrometer (MS-MS).

## Florida Department of Agriculture and Consumer Services Multi-Residue Method

The Florida laboratory uses an acetonitrile-based extraction with aminopropyl solid phase extraction (SPE) cleanup procedures to extract low-fat, high moisture commodities (i.e., fruit and vegetables) for pesticide screening. Fifty grams of homogenized sample is shaken with 50 mL acetonitrile. Sodium chloride is added to the sample/acetonitrile mixture and agitated. Samples for gas chromatography (GC) analyses are cleaned up using an aminopropyl SPE cartridge, eluted with 25% toluene in acetone and reconstituted in iso-octane for instrumental analysis. Samples for liquid chromatography (LC) analyses are cleaned up using an aminopropyl SPE cartridge, eluted with 3% methanol in acetone and reconstituted in methanol for instrumental analysis. Instrument/detector combinations used by Florida include GC/halogen specific detector (XSD), GC/flame photometric detector (FPD), GC/mass selective detector (MSD), GC/mass spectrometer (MS) ion trap, and LC/tandem mass spectrometer (MS-MS). Compounds analyzed include all major pesticide classes (e.g., organophosphates, organochlorines, carbamates, triazines, triazoles, pyrethroids, neonicotinyls, strobilurins

## Modified Luke Multi-Residue Method

One PDP laboratory (Ohio) uses a modified version of the Luke method[2,3] to extract fruit and vegetable samples for multi-residue method (MRM) pesticide screening. One hundred grams of homogenized sample is extracted with acetone. An aliquot of the filtrate is partitioned with methylene chloride and petroleum ether. Two additional methylene chloride partitions are performed. The final 10 mL extract is reconstituted in acetone and split for separate analysis using various instruments. Instrument/detector combinations used include gas chromatography (GC)-nitrogen/phosphorous detector (NPD), GC/electrolytic conductivity detector (ELCD), GC/flame photometric detector (FPD), GC/pulsed flame photometric detector (PFPD), GC/mass selective detector (MSD), and high performance liquid chromatography (HPLC) with post- and/or pre-column derivatization and fluorescence detector (FD).

---

[1] S.M. Lee, M.L. Papathakis, H.C. Feng, G.F. Hunter and J.E. Carr, **1991**, *Fres. J. Anal. Chem.* 339, 376-383

[2] Luke, M.A., *et al.*, **1975**, *J. Assoc. Off. Anal. Chem*,. 58, 1020-1026

[3] Luke, M.A., *et al.*, **1981**, *J. Assoc. Off. Anal. Chem*,. 64, 1187-1195

## New York Modified SPE Method

The New York Department of Agriculture and Markets laboratory uses a method based on the Agriculture and Agri-Food Canada solid phase extraction (SPE) method[4] with some improvements based on the Luke extraction[5]. Fifty grams of sample is extracted with 5% ethanol in acetonitrile and 15 grams sodium chloride. The organic layer is dried with sodium sulfate and cleaned up using a combination of SPE cartridges: graphitized carbon (Envi-carb), strong anion exchange (SAX), and weak anion exchange (PSA). Compounds are eluted with 3:1 mixture of acetonitrile:toluene, reconstituted in the appropriate solvent, and analyzed by the following instrument/detector combinations: gas chromatography (GC)/flame photometric detector (FPD), GC/tandem mass spectrometry (GC/MS/MS), and high performance liquid chromatography (HPLC)/tandem mass spectrometry (LC/MS/MS).

## QuEChERS Multi-Residue Method

The Michigan laboratory uses a modified version of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method[6]. The QuEChERS method was developed at the USDA Agricultural Research Service (ARS) Eastern Regional Research Center in Wyndmoor, Pennsylvania by Michelangelo Anastassiades, Steve Lehotay, et al to extract fruit and vegetable samples for multi-residue method (MRM) pesticide screening. Fifty grams of homogenized sample is extracted with acetonitrile. Either sodium chloride or a combination of sodium chloride and magnesium sulfate is added to the sample/acetonitrile mixture and agitated. The extract is then filtered or centrifuged and the organic layer is separated from the aqueous layer. The volume of the organic layer is reduced and an aminopropyl solid phase extraction (SPE) clean-up is employed prior to sample analysis. Instrument/detector combinations used include gas chromatography (GC)/halogen specific detector (XSD), GC/flame photometric detector (FPD), GC/pulsed flame photometric detector (PFPD), GC/mass selective detector (MSD), GC/tandem mass spectrometry (MS/MS), high performance liquid chromatography (HPLC) with post- and/or pre-column derivatization and fluorescence detector, liquid chromatography (LC)/MSD, and LC/MS/MS.

## Analytical Method for Pesticide Residue Testing in Meat Products

PDP has analyzed meat (beef and pork) and poultry using a multi-residue method (MRM) developed by the AMS National Science Laboratory (NSL). Approximately one pound of pork or poultry tissue (muscle or adipose) is homogenized with an equal amount of dry ice in a stainless steel blender and the ground sample is placed in a -20°C freezer to allow the dry ice to sublime. Ten grams of adipose tissue (or 20 grams of muscle tissue) is weighed and transferred to a 250 mL centrifuge bottle followed by the addition of 50 mL ethyl acetate. The suspension is mixed with a tissuemizer for one minute followed by the addition of four grams of magnesium sulfate and one gram of sodium chloride. After

[4] Fillion J., Hinde R., Lacroix J., Selwyn J., **1995**, *J. AOAC Int*,. 78, 1252-1266
[5] Luke M.A., Laboratory Information Bulletin, September 1994, Vol. 10, No. 9, Sec. 3896. Also previously published as "Luke, M.A., *et al.*, **1975**, *J. Assoc. Off. Anal. Chem*,. 58, 1020-1026" and "Luke, M.A., *et al.*, **1981**, *J. Assoc. Off. Anal. Chem*,. 64, 1187-1195".
[6] Anastassiades, M., Lehotay, S.J., Stajnbaher, D., and Schenck F., **2003**, *J. AOAC Int.*, 86, 412-431

briefly mixing, the samples are frozen at -20 ºC for approximately 30 minutes followed by centrifugation at 10,000 RPM for 5 minutes. The ethyl acetate layer is filtered into a 50 mL glass centrifuge tube and the volume adjusted to 20 mL with ethyl acetate. The extract is concentrated under a stream of nitrogen (in a 70 ºC bath) to a constant volume of 2 to 4 mL, diluted to 20 mL with acetonitrile, vortexed for one minute, and frozen at -70 ºC for 30 minutes. The frozen extract is centrifuged for 3 minutes at 10,000 RPM and the acetonitrile layer is filtered using a 0.45 µm syringe filter into a 15 mL glass centrifuge tube and allowed to come to room temperature. The volume is adjusted to 15 mL with acetonitrile and concentrated to 1.5 mL under a stream of nitrogen in a 70 ºC water bath. The extract is transferred to a 2 mL mini-centrifuge vial containing 50 mg of PSA sorbent, 50 mg of C18 sorbent, and 150 mg of magnesium sulfate, and vortexed for 30 seconds followed by centrifugation for one minute. The extract is syringe-filtered using a 0.25 µm filter into 2 mL autosampler vials for analysis by gas chromatography (GC)/mass spectrometry (MS) and liquid chromatography (LC)/MS/MS.

Instrument Parameters:

GC/MS: Agilent 6890 GC with Agilent 5975 Mass Selective Detector
Column: J&W Scientific DB-5MS w/ Duraguard - 30 M length x 0.25 mm ID x 0.25 µm film
Carrier gas: Helium
Injection port temperature: 240 ºC
Injection port liner: Agilent #5183-4694 single gooseneck with glass wool
Injection volume: 1 µL
Flow: constant flow at 1.3 mL/min with post-run flow of 2 mL/min
Oven program: Initial temp 70 ºC; hold 2 min
20 ºC/min to 170 ºC hold 0 min
5 ºC/min to 220 ºC hold 0 min
10 ºC/min to 312 ºC hold 0 min
Post-run time – 3.8 min @ 325 ºC

LC/MS/MS: Agilent 1100 LC with Thermo Quantum Discovery Max Triple Quadrupole Mass Spectrometer
Column: Zorbax C18 2.1 mm width x 150 mm length x 3.5 µm particle size
Mobile phase: 5 mM ammonium acetate in water (mobile phase A)
95/5 methanol / 5mM ammonium acetate in water (mobile phase B)
Injection volume: 5 µL
Flow: 0.25 mL/min

| Gradient: | | | |
|---|---|---|---|
| | 0-5 min | 85% A | 15% B |
| | 5-25 min | 60% A | 40% B |
| | 25-29 min | 10% A | 90% B |
| | 29-38 min | 85% A | 15% B |

## PDP Analytical Method for Pesticide Residue Testing in Dairy Products

PDP has analyzed milk, cream, and butter using a multi-residue method (MRM) developed by the AMS National Science Laboratory (NSL). Approximately 100 grams milk, 10 grams cream, or 5 grams butter is accurately weighed and transferred to a 250 mL centrifuge bottle followed by the

addition of 100 mL ethyl acetate (for milk, 90:10 ethyl acetate:ethanol). The suspension is agitated for 5 minutes on a wrist action shaker followed by the addition of 40 grams of magnesium sulfate and 10 grams of sodium chloride to milk samples or 4 grams of magnesium sulfate and 1 gram of sodium chloride to cream or butter samples. After briefly mixing the samples are cooled for approximately 30 minutes in an ice water bath followed by centrifugation at 10,000 RPM for 5 minutes.

The ethyl acetate layer is filtered into a 50 mL glass centrifuge tube and the volume adjusted to 50 mL with ethyl acetate. The extract is then concentrated under a stream of nitrogen in a 70 ºC bath to a constant volume of approximately 1.5 to 2 mL. It is diluted to 25 mL with acetonitrile, vortexed for one minute, and frozen at -70 ºC for 30 minutes. The frozen extract is centrifuged for 3 minutes at 10,000 RPM and the acetonitrile layer is filtered through a 0.45 µm syringe filter into a 15 mL glass centrifuge tube and allowed to come to room temperature. The volume is adjusted to 15 mL with acetonitrile and concentrated to 1.5 mL under a stream of nitrogen in a 70 ºC water bath and the extract is transferred to a 2 mL mini-centrifuge vial containing 50 mg of PSA sorbent, 50 mg of C18 sorbent, and 150 mg of magnesium sulfate, and vortexed for 30 seconds followed by centrifugation for one minute. The extract is syringe-filtered through a 0.25 µm filter into 2 mL autosampler vials for analysis by gas chromatography (GC)/pulsed flame photometric detector (PFPD), GC/electron capture detector (ECD), GC/mass selective detector (MS), and liquid chromatography (LC)/post-column derivatization (PCX)-fluorescence detector (FLD).

GC/PFPD: Agilent 6890 GC with dual OI Analytical 5380 Pulsed Flame Photometric DetectorsPFPDs
Columns: Front – Restek Rtx-1 with Ultraguard 30 M length x 0.32 mm id x 0.5 µm film thickness
Back – Restek Rtx-OPP2 30 M length x 0.32 mm id x 0.32 µm film thickness
Carrier gas: Helium
Injection port temperature: 250 ºC
Injection port liner: Agilent #5183-4694 single gooseneck with glass wool
Injection volume: 2 µL
Flow: Constant flow at 1.8 mL/min with post-run flow of 12 mL/min
Detector: Dual PFPDs in phosphorous mode
Oven program: Initial temp 70 ºC; hold 1 min
15 ºC/min to 200 ºC; hold 0 min
4 ºC/min to 250 ºC; hold 0 min
10 ºC/min to 300 ºC; hold 3.5 min
Post-run time – 6 min at 300 ºC

GC/ECD: Agilent 6890 GC with dual Electron Capture Detectors
Columns: Front – J&W Scientific DB-35 30 M length x 0.32 mm id x 0.5 µm film thickness
Back – Restek Rtx-CLP2 30 M length x 0.32 mm id x 0.25 µm film thickness
Carrier gas: Helium
Injection port temperature: 250 ºC
Injection port liner: Agilent #5183-4694 single gooseneck with glass wool
Injection volume: 1 µL
Flow: Constant flow at 2.0 mL/min with post run flow of 12 mL/min
Detector: Dual ECDs

Oven program: Initial temp 80 ºC; hold 1 min
15 ºC/min to 200 ºC; hold 0 min
5 ºC/min to 300 ºC; hold 15 min
Post-run time – 6 min at 305 ºC

GC/MS: Agilent 6890 GC with Agilent 5973 Mass Selective Detector
Column: J&W Scientific DB-5MS w/ Duraguard - 30 M length x 0.25 mm id x 0.25 µm film thickness
Carrier gas: Helium
Injection port temperature: 250 ºC
Injection port liner: Agilent #5183-4694 single gooseneck with glass wool
Injection volume: 1 µL
Flow: Constant flow at 1.0 mL/min with post run flow of 2 mL/min
Oven program: Initial temp 80 ºC; hold 2 min
10 ºC/min to 120 ºC; hold 0 min
3 ºC/min to 240 ºC; hold 0 min
10 ºC/min to 300 ºC; hold 0 min
Post-run time – 8 min at 325 ºC

LC/PCX-FLD: Agilent 1100 LC with fluorescence detection and post-column derivatization
Post-column derivatization unit: Pickering PCX5200
Reagent flow: 0.2 mL/min
Reagent 1: 0.05 N NaOH in water
Reagent 2: OPA reagent (0.14 g OPA & 2 mL 2-mercaptoethanol in 1 L of 0.05 potassium tetraborate tetrahydrate)
Column: Pickering C18 4.6 mm width x 250 mm length x 5 µm particle size
Column temp: 42 ºC
Injection volume: 10 µL
Flow: 0.8 mL/min
Detector: Fluorescence – excitation at 330 nm and emission at 455 nm
Mobile phase: Deionized water (mobile phase A)
Methanol (mobile phase B)

| Gradient: | | | |
|---|---|---|---|
| | 0-1 min | 85% A | 15% B |
| | 1-44 min | 25% A | 75% B |
| | 44.1-49 min | 0% A | 100% B |
| | 49.1-57 min | 85% A | 15% B |