Sections in This Document

1. Purpose

This document presents a training protocol about minimum provisions for preparation of supportive pesticide regulatory analytical packages.

2. Scope

This document applies to analysts, technicians, and supervisors who work on pesticides analyses for regulatory purposes.

3. Responsibility

A. Supervisor
   1. Ensure personnel have access to this document and any necessary training
   2. Ensure personnel have the materials and instrumentation necessary to complete samples to the necessary requirements

B. Analyst/Technician
   1. Follow the requirements described in this document
   2. Bring concerns to the supervisor’s attention

4. Background

The statutory authority for control of pesticide residues in food began with the Food and Drug Act of 1906 and was strengthened with passage of the Federal Food, Drug, and Cosmetic Act (FD&C) of 1938. In 1954, the Pesticide Chemical Amendment (Public Law 518) was passed. The amendment provided a new, more effective procedure for controlling residues of pesticide chemicals used on raw agriculture commodities (which include dairy products, eggs, and fish). The Secretary was given authority to establish tolerances in or on commodities and to require petitions from manufacturers requesting the establishment of tolerances. Data required in these petitions include name, composition, and chemical identity of the pesticide and its residues; residue data from actual applications of the pesticide; toxicity studies; and analytical methods for determining residues. Data requirements and a summary of FDA activities in this area can be found in "FDA Papers," Vol. 2, No. 7, September 1968.

In 1970, the function of establishing tolerances for pesticide chemicals was transferred to the Environmental Protection Agency (EPA). FDA continues to have regulatory responsibility for enforcing the tolerances.

Under the FD&C Act, a raw agricultural commodity is considered adulterated if it bears or contains a pesticide chemical that is "unsafe" within the meaning of section 408. A chemical is "unsafe" unless its quantity is within limits of

prescribed tolerances or unless it has been exempted from the tolerance requirement. Tolerances and exemptions, based on usage, stability, and toxicological properties of the residue, are found in 40 CFR Part 180.

The earliest analytical methods were procedures based on classic elemental analysis, spectrophotometric, biochemical, and biological methods. During the 1950s and early 1960s, chromatographic methods, with greater sensitivity and the capability of distinguishing residues within a given class, were introduced. As a result of constantly improving analytical procedures, "negligible residue" tolerances, defined in 40 CFR Part 180.1(1), are now established for selected crops and residues. "Negligible residue" tolerances replaced the prior concept of "zero" tolerance for chemicals of high toxicity.

Analytical procedures, compiled in the FDA Pesticide Analytical Manual (PAM) and the Official Methods of Analysis of the AOAC, demand a high level of analytical skill and technique to provide meaningful regulatory results. Currently, FDA's Office of Regulatory Affairs (ORA) labs use a harmonized multi-residue pesticide method that uses a modified QuEChERS (Quick Easy Cheap Effective Rugged Safe) extraction coupled with chromatography-mass spectrometry based detection. This harmonized method has been multi-lab validated. For check and/or additional analyses, and for additional analytical work, past the initial screening, ORA labs can use different procedures that can remove interfering compounds and matrices, investigate novel findings, and quantify challenging residues. Similarly, consumer complaints and follow-up samples can demand different analytical procedures. There are protocols established by the FDA foods program that outline how method modifications, qualifications and validations can be performed when changes to methods occur or when new methods are needed to address different analytical situations. The trainee should develop familiarity with the Foods Program Methods Validation Processes and Guidelines. To develop the necessary analytical skills, the trainee will perform the exercises in this section, which follow the harmonized pesticide method protocol, and advanced chromatographic and mass spectrometric techniques, that are employed in pesticide analysis for the separation of complex sample mixtures. To demonstrate proficiency in this area, the trainee will analyze both regulatory and fortified samples. The trainer will assign the exercises.

Finally, the trainer will discuss the current revision of SOP-000500 Determination of Pesticides and Industrial Chemicals Using Modified QuEChERS and GC-MS/MS and LC-MS/MS and WI-000292 Guidance for the Analysis, Documentation and QA/QC processes to Support Regulatory Action in the FDA/ORA Pesticide Program.

5. References

A. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed Emerita). (1994). Pesticide Analytical Manual Vol. I, section 205-*Reference Standards*, updated October 1999). R.O.W. Sciences, Inc.
B. Laboratory Information Bulletin, LIB No. 633 and LIB No.1386.
C. Hodgson, D. W., Thompson, J. F., Watson, R. R. (1982). *Journal of Association of Official Analytical Chemists*, 65, 89-93; 94-102.
D. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, chap. 5-Gas Liquid Chromatography, updated October 1999). R.O.W. Sciences, Inc.
E. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, section 501-General Information, updated October 1999). R.O.W. Sciences, Inc.
F. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed Emerita). (1994). Pesticide Analytical Manual Vol. I, section 302-*Multiclass multiresidue methods*, updated October 1999). R.O.W. Sciences, Inc.
G. Internal laboratory GC methods and SOPs.
H. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed. Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, section 105-Analytical Limits of Quantitation, updated October 1999). R.O.W. Sciences, Inc.
I. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed. Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, sections 302-Mehtod I for Nonfatty Foods and 303-Method II for nonfatty Foods, updated October 1999). R.O.W. Sciences, Inc.

J. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed. Emerita). (1994). Pesticide Analytical Manual-*multiresidue methods* (3rd ed., Vol. I, section 102-Preparation of Analytical Samples, updated October 1999). R.O.W. Sciences, Inc.
K. U.S. Food & Drug Administration, Office of Regulatory Affairs, (current edition). Investigations operations manual (IOM) (chap. 4, Sample Schedule Chart 3 for Pesticides, pp. 196-199).
L. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed. Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, section 304-Method for Fatty Foods, updated October 1999). R.O.W. Sciences, Inc.
M. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed. Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, Appendix I, PESTDATA, updated October 1999). R.O.W. Sciences, Inc.
N. FACTS reporting manual.
O. Pesticide Glossary.
P. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed. Emerita). (1994). Pesticide analytical manual-*multiresidue methods* (3rd ed., Vol. I, (updated October 1999). R.O.W. Sciences, Inc. section 504- Quantitation, (updated 10/99) R.O.W. Sciences, Inc.
Q. 40 C.F.R. Part 136 Appendix B Revision 2
R. Foods Program Methods Validation Processes and Guidelines

6. Procedure

6.1. Equipment, Supplies, and Reagents

6.1.1. Objective

To familiarize the trainee with the pesticide residue laboratory and its equipment.

6.1.2. Assignment

The trainer will discuss the location, care, and cleaning procedures for equipment found in the residue laboratory and will explain proper procedures to obtain, handle, and store needed reagents and solvents. The harmonized multi-residue pesticide method will be reviewed. (SOP-000500 Determination of Pesticides and Industrial Chemicals Using Modified QuEChERS and GC-MS/MS and LC-MS/MS)

Reading and reference materials pertinent to pesticides will be provided.

6.1.3. Reference

U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Plant, Dairy Foods, and Beverages. Makovi, C. M. (Ed.), McMahon, B. M. (Ed Emerita). (1994).

Pesticide Analytical Manual-multiresidue methods (3rd ed., Vol. I, Chaps. 1-Regulatory Operations and 2-General Analytical Operations and Information, updated October 1999). R.O.W. Sciences, Inc.

6.2. Standard Solutions

6.2.1. Objective

To introduce the subject of pesticide standards: proper procedures for preparation, precautions during preparation and use, and problems associated with degradation.

6.2.2. Assignment

The trainer will explain the laboratory standard operating procedure (SOP) for preparing "stock" and "working" standard solutions, including documentation of standard solution preparation and comparison to previous standards. Proper storage of primary and working standards and associated problems, such as degradation or toxicity, will be discussed.

1. Determine the proper handling of toxic compounds.
2. Read the references and answer the questions that follow. Use this procedure for all subsequent assignments. Optional assignments will be completed as needed by the trainee.
3. Determine procedures for ordering standards from commercial vendors and EPA.

6.2.3. Questions

1. Which SOP is used in the laboratory to ensure primary standard purity and reliability?

2. What constitutes a stock solution? A working solution?
3. Which SOP is used to ensure reliability of stock and working solutions?
4. What are the hazards associated with handling and storage of pesticide standards?
5. What class of pesticides is particularly toxic by skin absorption as well as by inhalation and ingestion?
6. Where can information such as chemical structure for EPA standards be found?

6.3. Gas Chromatography (In labs that have standalone GCs)

6.3.1. Instrumentation and Apparatus

A. Objective
To present an overview of basic gas chromatographic (GC) apparatus in the laboratory.

B. Assignment
The trainer will introduce basic gas chromatographic apparatus for the various commercial units in the laboratory and explain its use in pesticide analysis.

C. Instruction
Using a block diagram, illustrate the basic GC apparatus needed for pesticide analysis.

6.3.2. Injection Techniques

A. Objective
Autosamplers are used exclusively to inject.

B. Assignment
The training will include proper syringe handling and cleaning, and a discussion of injection volume.

C. Questions

1. What is the major consideration in using the "solvent flush" technique for GC injections?
2. Generally, what causes bubbles or non-smooth draw up of solvent into a syringe?
3. What are the practical volumes to inject using a 10 µL syringe? What is the desirable percentage relative standard deviation for repeated injections?

4. What effects can be expected from a leaky septum?
5. Explain the "blow-by" phenomenon.
6. How is a syringe checked for "blow-by?"
7. Explain available automated injection techniques e.g. split, split/spitless, on-column, and temperature programming.

6.3.3. Columns

A. Objectives

1. To present techniques and precautions for preparing columns; utilization of capillary columns and injection systems.
2. To evaluate the separation characteristics of the commonly used pesticide columns.
3. To obtain an understanding of performance criteria for acceptable GC columns.

B. Questions and Instructions

1. What GC parameter has the greatest effect on retention times?
2. What is retention time locking and how is it used?
3. What are some of the indicators of a deteriorating column? How can column performance be improved?
4. Describe the effect of pesticide polarity on chromatography.

6.4. Single Quadrupole Mass Spectrometry (MSD)

6.4.1. Objective

To train in proper instrument startup, and to introduce the fundamental principles, software, uses, NIST library search and safe handling the instrument.

6.4.2. Assignment

The trainer will demonstrate the following:

1. Starting up and shutting down the single quadrupole mass spectrometer (MSD) including the pump down and venting procedures.
2. Vacuum integrity using the Air and Water Check procedure.
3. Tuning the MSD using the manual and autotune procedures.
4. Operating the MSD in the full Scan Mode.
5. Identifying compounds using the Library Search routines.

6. Operating in the Selected Ion Monitoring (SIM) mode, including selection of ions to be monitored, dwell times and retention time windows.
7. Preparing an MSD analytical sequence for a SIM Method.
8. Analyzing data generated by a SIM analytical run, including verifying the identification of compounds, and quantification of detected compounds.

6.4.3. Questions

1. Why should an MSD with a diffusion pump be cooled below 100º C before venting?
2. What can we learn from the ratio of air to water ions in the Air and Water Check?
3. What is the highest electron multiplier voltage setting?
4. In the full scan mode, what is the mass to charge range available?
5. Describe the libraries available using the data system.
6. What are the tradeoffs between the following: dwell time, number of ions monitored in a SIM Method and the shape of the chromatographic peak?
7. Why is the start operation of the MSD delayed? (i.e. a few minutes after the sample is injected).

6.5. Triple Quadrupole Mass Spectrometry (QQQ)

6.5.1. Objective

To train in proper instrument startup, and to introduce the fundamental principles, software, uses, and safe handling the instrument.

6.5.2. Assignment

Following trainer demonstration, start up a designated triple quadrupole mass spectrometer (QQQ) system and inject pesticide standard mixture. The trainer will demonstrate the following:

1. Starting up and shutting down the triple quadrupole mass spectrometer (QQQ) including the pump down and venting procedures.
2. Vacuum integrity using the Autotune/Air and Water Check procedure.
3. Tuning the QQQ using the manual and autotune procedures.
4. Analyzing data generated by a MRM analytical run, including verifying the identification of compounds, and quantification of detected compounds.

6.5.3. Questions

1. How is a triple quadrupole mass spectrometer (QQQ) different from a single quadrupole mass spectrometer (MSD)?
2. What scan modes are possible when using a QQQ? What scan mode is most used for multiresidue pesticides analysis?
3. What are the main advantages of a QQQ over other mass spectrometric detectors?
4. What is backflushing and what purpose does it serve?

6.6. Liquid Chromatography-Mass spectrometry

A. Objective

To present an overview of basic liquid chromatography instrumentation and apparatus in the laboratory.

B. Assignment

The trainer will introduce basic liquid chromatographic apparatus for the various commercial units in the laboratory and explain its use in pesticide analysis. The locations of components, including mobile phases, degasser, autosampler, pumps, columns, detectors, and data systems will be shown and discussed.

C. Questions

1. How do we use aqueous and organic solvents as mobile phases for high-performance liquid chromatography (HPLC)?
2. How does pH of the mobile phase affect the separation of compounds?
3. How do air bubbles change the results of HPLC?
4. How does small leakage affect the results of HPLC?
5. What factors can change the back pressure?

6.6.1. HPLC Columns

A. Objective

1. To introduce some representative high-performance liquid chromatography (HPLC) columns commonly used in pesticide residue analysis.
2. To present techniques and precautions for stationary phases and differences between HPLC and ultra-high-performance liquid chromatography (UHPLC or UPLC).

3. To evaluate the separation characteristics of the commonly used pesticide columns.
4. To obtain an understanding of performance criteria for acceptable HPLC columns.

B. Assignment

Discuss performance criteria for acceptable HPLC columns.

C. Questions

1. How does a C18 column separate the compounds in HPLC?

6.7. Liquid Chromatograph with Triple Quadrupole Tandem Mass Spectrometry (LCMS or LC-MS/MS)

A. Objective

To train in proper instrument startup, and to introduce the fundamental principles, software, uses, and safe handling the instrument.

B. Assignment

The trainer will demonstrate the following:

1. Starting up and shutting down the LC-MS/MS including the pump down and venting procedures.
2. Operating the LC-MS/MS in the multiple reaction monitoring (MRM) Mode.
3. Operating in the scheduled MRM (sMRM) mode, including MRM(s) to be monitored, dwell times and retention time windows.
4. Preparing a LC-MS/MS analytical sequence for a sMRM method.
5. Analyzing data generated by a sMRM or MRM analytical run, including screening and quantitation of compounds.

C. Questions

1. Why it is important to stop the turbo pump before turning the power off?
2. How do we tell if an LC-MS/MS problem is due to HPLC or tandem mass spectrometry?
3. What are the trade-offs between the following: dwell time, number of ions monitored in an MRM Method and the number of data points in a chromatographic peak?

4. Why is the divert valve used between HPLC and tandem mass spectrometry?

6.8. Quantification

A. Objectives

1. Introduce general concepts for measuring gas chromatographic peaks used in routine quantification of pesticide analytes.
2. Introduce the strategy for quantification of complex mixtures or multicomponent compounds.
3. Instrument Specific:

a. LC-MS/MS determination (Liquid chromatography): Quantitate residues using standard addition with minimum of 3 standard additions; OR a calibration curve of standards prepared in the same matrix (r2>0.99 for 5 calibration points).

b. GC-MSD and GC-MS/MS determination (gas chromatography): Quantitate residues using a single concentration standard, prepared in an appropriate matrix, that is within 5X of the incurred residue response; OR standard addition with minimum of 3 standard additions.

B. Assignment

Under a trainer's guidance, make standard and sample injections of various analytes, including mixtures, perform the appropriate calculations and discuss the results completely.

C. Questions

1. Why are internal standards used? What is an ideal internal standard? How are internal standards used in quantitation?
2. Why is it important to review the chromatograms when performing quantitation?
3. What are qualifier transitions and how do they differ from quantifier transitions?
4. How can issues such as split peaks or inadequate integration be addressed?

6.9. Sample Analysis

A. Method Detection Limit (MDL)

40CFR136 Appendix B defined the method detection limit:

The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.

(1) The MDL procedure is designed to be a straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods. The procedure requires a complete, specific, and well-defined analytical method. It is essential that all sample processing steps used by the laboratory be included in the determination of the method detection limit.

(2) The MDL procedure is not applicable to methods that do not produce results with a continuous distribution, such as, but not limited to, methods for whole effluent toxicity, presence/absence methods, and microbiological methods that involve counting colonies. The MDL procedure also is not applicable to measurements such as, but not limited to, biochemical oxygen demand, color, pH, specific conductance, many titration methods, and any method where low-level spiked samples cannot be prepared. Except as described in the addendum, for the purposes of this procedure, “spiked samples” are prepared from a clean reference matrix, such as reagent water, spiked with a known and consistent quantity of the analyte. MDL determinations using spiked samples may not be appropriate for all gravimetric methods (e.g., residue or total suspended solids), but an MDL based on method blanks can be determined in such instances.

It is often used to estimate the limit if detection (LOD) and the limit of quantitation (LOQ).

B. Objective

To acquaint the trainee with method detection level (MDL) significance and determination.

C. Assignment

Read the reference and become familiar with its contents (40 CFR 136 Appendix B).

D. Questions

1. For what types of methods can an MDL be calculated? Do pesticides methods qualify?
2. Give one way an MDL can be estimated prior to performing lab work.

6.10. Sample Preparation and Compositing

A. Objective

To familiarize the trainee with Pesticide Analytical Manual (PAM) | FDA and QuEChERS methods for sample preparation and compositing.

B. Assignment

The trainer will explain how to prepare the following samples and how to select a representative portion to be held by the laboratory:

1. A case of apples and a case of peaches
2. Smelt sample consisting of 5 fish, less than 2 lb total
3. Milk concentrate and low-fat dairy product
4. As assigned, prepare samples for laboratory analysis.

C. Questions

1. How would one prepare a sample if 20 melons are submitted to the laboratory?
2. How would one prepare a sample of six l-gallon cartons of milk?
3. How would one prepare a sample of 20 lb of wheat?
4. How would one prepare a sample of slightly decomposed heads of lettuce? Very decomposed heads of lettuce?
5. What are the major considerations for a laboratory sample regarding the sample received by the laboratory and storage of reserve portions?

6.11. Nonfatty Foods-Multiresidue Determination

A. Objective

To acquaint the trainee with methods used to prepare, extract, and clean up samples of nonfatty foods for analysis of pesticide residues.

B. Assignment

1. Under the observation of an experienced analyst, prepare, extract, and clean up routine samples, including a fortified (spiked) sample and a reagent blank.
2. Store all sample extracts for GC MS and LCMS determination until completion of the next section on fatty foods.

C. Questions and Instructions

1. Briefly describe the extraction and cleanup principles involved in this assignment.

2. What salts are used in this QuEChERS extraction and what function does each perform?
3. How does the extraction diverge for LC and GC extracts?

6.12. Fatty Foods-Multiresidue Determination

A. Objective

To acquaint the trainee with methods used to prepare, extract, and clean up samples of fatty foods for analysis of residues.

B. Assignment

Under the observation of an experienced analyst, prepare, extract, and clean up several routine samples, a reagent blank, and a fortified sample (milk or cheese is suggested).

C. Questions

1. Briefly describe the extraction and cleanup principles involved in the assignment. How is the extraction different from the high moisture procedure performed above?
2. What is the purpose of the addition of water to the fatty samples?

6.13. Sample Results, Evaluation, and Reporting

A. Objective

To evaluate gas chromatograms and identify pesticide residues.

B. Assignment

1. Under the trainer's observation, put the correct GC instrumentation into operation for multiresidue determination of the previously prepared samples.
2. Submit daily chromatograms demonstrating proper analyte separation and instrumentation sensitivity, according to laboratory SOP.

6.14. Pesticide Residue Confirmation

A. Objective

To provide training for the identification and quantification of pesticide residues in FDA regulated commodities. In addition, this training is designed to provide detailed information required to properly assemble a data package capable of supporting regulatory action.

B. Assignment

Read SOP-000500 Determination of Pesticides and Industrial Chemicals Using Modified QuEChERS and GC-MS/MS and LC-MS/MS and WI-000292 Guidance for the Analysis, Documentation and QA/QC processes to Support Regulatory Action in the FDA/ORA Pesticide Program. The trainer will then review in detail all the required elements to generate an analytical package.

C. Questions

1. Where do you find the official tolerances for pesticide residues listed?
2. When the level of a residue exceeds an official tolerance, what type of analysis is performed? How close must this agree with the original analysis?
3. If there is no tolerance for a residue, what value must the analyte level exceed?
4. In the identification of residues, what is required to “Confirm” the identity of an analyte?
5. When is recovery information required?
6. Is quantification always required on Original and Check analyses?

7. Glossary/Common analytical Acronyms used

A. **GC**: Gas Chromatography
B. **GC-QQQ**: Gas Chromatograph Triple Quadrupole Mass Spectrometer.
C. **HPLC**: High Performance Liquid Chromatography
D. **LC**: Liquid Chromatography
E. **LCMS** or **LC-MS/MS**: Liquid Chromatograph with Triple Quadrupole Tandem Mass Spectrometry
F. **MRM**: Multiple Reaction Monitoring
G. **MSD**: Single Quadrupole Mass Spectrometer
H. **MS-QQQ**, **Triple Quadrupole MS**, or **QQQ** refer to Triple Quadrupole Mass Spectrometer
I. **QuEChERS**: Quick Easy Cheap Effective Rugged Safe
J. **UHPLC** or **UPLC**: Ultra-High-Performance Liquid Chromatography

8. Supporting Documents

A. Pesticide Analytical Manual (PAM) | FDA
B. SOP-000500 Determination of Pesticides and Industrial Chemicals Using Modified QuEChERS and GC-MS/MS and LC-MS/MS

C. WI-000292 Guidance for the Analysis, Documentation and QA/QC processes to Support Regulatory Action in the FDA/ORA Pesticide Program.

9. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
1.2	R	09/01/2005	LMEB	LMEB
1.3	R	02/13/2014	LMEB	LMEB
02	R	01/18/2021	LMEB	LMEB
03	R	REFER TO QMIS	ORS PESTICIDES HARMONIZATION WORKGROUP	LMEB

* - D: Draft, I: Initial, R: Revision

10. Change History

Revision #	Change
1.2	Sections 5.4.5, 5.4.6, 5.5, 5.7, 5.8, 5.10 added
1.3	Header – Division of Field Science changed to Office of Regulatory Science 5.1, 6th paragraph – revised to ORA-LAB.010 5.6.2. C. – removed Division name 5.8 B. and C, – revised to ORA-LAB.010
02	• Comprehensive revision by Pesticides Harmonization and Standardization Committee to reflect current practices. • Revised formatting and content outline
03	• Removed all references to ORA-LAB.010 (archived). Added replacement documents: SOP-000500 (Method), WI-000292, Guidance for the Analysis, Documentation and QA/QC processes to Support Regulatory Action in the FDA/ORA Pesticide Program. Added current use of QuEChERS information to Background section. Added meaning of method acronyms used.

11. Attachments

List of Attachments

Attachment A - Answer Key

6.2.3 Standard Solutions

1. **Which SOP is used in the laboratory to ensure primary standard purity and reliability?**
   (Quote local laboratory pesticide standard SOP.) PAM I, section 205 addresses pesticide reference standards.
2. **What constitutes a stock solution? A working solution?**
   A stock solution is the initial solution from which other diluted solutions are prepared. Working solutions are prepared from the stock solutions and further diluted for use in quantitation. (PAM I, 205-3)
3. **Which SOP is used to ensure reliability of stock and working solutions?**
   (Quote local laboratory pesticide standard SOP.) PAM I, section 205 addresses pesticide reference standards.
4. **What are the hazards associated with handling and storage of pesticide standards?**
   All pesticides are toxic, but the relative degree of toxicity varies greatly. Some are readily absorbed through the skin, especially the liquids. Skin absorption is enhanced by dilution in solvents when preparing standard solutions. Gloves should be used when handling and preparing pesticide standards. Another entry route is inhalation; therefore, pesticide standards should be handled inside a safety hood. Studies with rats indicate that pregnant women have increased susceptibility to pesticide poisoning, therefore should not handle pesticide standards. The Material Safety Data Sheet supplied with each reference standard contains information about the toxicity, hazards, and safe handling of the compound. (LIB 663)
5. **What class of pesticides is particularly toxic by skin absorption as well as by inhalation and ingestion?**
   Organophosphate pesticides such as parathion are particularly toxic and absorb through the skin rapidly. (LIB 663)
6. **Where can information such as chemical structure for EPA standards be found?**
   Structural information for pesticides can be found in several resources including the Merck Index, Farm Chemicals Handbook, Agrochemicals Handbook, FDA Surveillance Index, Menzie's Metabolism of Pesticides, various chemical vendor catalogues, and via internet search.

6.3.2 Injection Techniques

1. **What is the major consideration in using the "solvent flush" technique for GC injections?**
   This technique when performed manually is the most reproducible among analysts

because the actual sample volume injected is measured and completely delivered regardless of individual differences. Another consideration is the amount of solvent used to flush the sample volume. Excessive solvent flush can distort the chromatography of the analytes, especially the earlier elutes. This effect can be eliminated using temperature programming to concentrate the analytes on the front of the column until the solvent is evaporated out of the injection port.

2. **Generally, what causes bubbles or non-smooth draw up of solvent into a syringe?**
   A vacuum is created in the syringe when the plunger is withdrawn too quickly.

3. **For acceptable practice, what are the practical volumes to inject using a 10 µL syringe? What is the desirable percent relative standard deviation for repeated injections?**
   For a 10 µL syringe, the practical injection volume is 3-8 µL. The relative standard deviation (RSD) of five injections of a 1 ppm standard should be 2 % or less. Greater RSD values can be expected for injections of lower concentrations.

4. **What effects can be expected from a leaky septum?**
   Leaky septa cause inaccuracies in quantitation, problems with chromatography, and exposure of the system to air. (PAM I, 502- 18)

5. **Explain the "blow-by" phenomenon.**
   If a syringe plunger does not seal against the syringe body properly the carrier gas of a GC can “blow by” the plunger out the back of the syringe.

6. **How is a syringe checked for "blow-by?”**
   Draw up 50 % of the syringe volume followed by a small volume of air, then insert the syringe into a GC, holding steadily and not allowing the plunger to move. If the air bubble moves toward the back of the syringe, the integrity of the syringe is compromised, and the syringe should be repaired or replaced.

7. **Explain automated injection techniques e.g. split, split/splitless, on-column, and temperature programming.**
   For split injections, the flow of the carrier gas is split at the injection port between the column and waste. The split ratio of column/waste is set with a valve in the injection port. This procedure is used for injections of highly concentrated analytes. Split/splitless injection ports are simply split injection ports with the ability to close the split so that the whole injection is introduced onto the column. For on-column injections, the injection needle is inserted into the column during injection. This technique calls for very low volume injections (1-2 µL) or temperature programming to prevent injection losses during rapid expansion of the sample solvent to vapor. Temperature programming allows the analyst to program the temperature of the injection port. The primary advantage of this technique is the solvent can be evaporated out of the injection port before the analytes are introduced onto the column eliminating undesirable solvent broadening effects.

6.3.3 Columns

1. **What GC parameter has the greatest effect on retention times?**
   Column temperature has the greatest effect on retention time. (PAM I, 502-4)
2. **What is retention time locking and how is it used?**
   Retention time locking is a means to normalize retention times when column conditions change. The retention time of a compound is monitored over multiple runs while the initial pressure or flow rate is incrementally changed. The resulting retention times are plotted along with the flow rates/pressures and an equation for the line is produced (not usually linear). From this equation, the flow rate/pressure required to give the ideal retention time is determined.
3. **What are some of the indicators of a deteriorating column? How can column performance be improved?**
   Peak broadening, tailing, reduced theoretical plates and capacity factors, and diminished resolution between adjacent analyte peaks are all signs of a deteriorating column. (PAM I, 502-4). In some cases, cutting a small amount off the front or back of the column can improve column performance. Changing method conditions (temperature maximums, backflushing procedures) can improve future column wear but is unlikely to improve an already deteriorating column.
4. **Describe the effect of pesticide polarity on chromatography and on column selection.**
   Polar pesticides tend to react with active silanol sites in the injection port and column causing peak broadening, tailing, and even splitting. Polar compounds chromatograph best on polar columns. (PAM I, 502-21)

6.4 Single Quadrupole Mass Spectrometry

1. **Why should an MSD with a diffusion pump be cooled below 100º C before venting?**
   The system can become contaminated if it is not cooled before venting.
2. **What can we learn from the ratio of air to water ions in the Air and Water Check?** When a system is initially pumped down the air pumped out more quickly than water; water tends to stick to the stainless steel. If the air is still high after a period, there may be a leak.
3. **What is the highest electron multiplier voltage setting?**
   Most electron multipliers have a limit of 3000 volts. They are normally operated in 1100 – 2500 volts range, with the voltage increasing during its lifetime to achieve good sensitivity. At higher voltages, system noise rises, limiting sensitivity.
4. **In the full scan mode, what mass to charge range (M/Z) is available?**
   The M/Z range can vary from instrument to instrument but is usually two to 800 atomic mass units (amu). It is useful to limit the scan range of a full scan to above

40 amu (to avoid ions of argon in the system) and below the largest molecular weight expected to be encountered (for pesticides, below 500 amu).

5. **Describe the libraries available using the data system.**
   The NIST Spectral Library is usually purchased with the instrument. Other spectral libraries, such as a pesticide library, are available from vendors. Become familiar with all the libraries on your system.
6. **What are the tradeoffs between the following: dwell time, number of ions monitored in a SIM Method and the shape of the chromatographic peak?**
   Long dwell times or many ions monitored will result in fewer data points taken across the GC eluting peak. This may result in misshapen peaks which can give poor quantification areas.
7. **Why is the start operation of the MSD delayed? (i.e. a few minutes after the sample is injected).**
   This will prevent the filament from burning out.

6.5 Triple Quadrupole Mass Spectrometry

1. **How is a QQQ different from an MSD?**
   A QQQ has three quadrupole mass filters. An MSD has one quadrupole mass filter/analyzer. This gives the QQQ more flexibility in the type of analyses that may be performed.
2. **What scan modes are possible when using a QQQ? What scan mode is most commonly used for multiresidue pesticides analysis?**
   A full scan (two of the quads pass the ions through and the last scans through the mass range for separation and detection); product ion scan (one ion is selected in the first quad, fragmented in the second quad, and the products are separated in the third quad); precursor scan (ions are scanned through quad one, fragmented in quad two, and only a certain product ion is selected in quad three); neutral loss (quads one and three are scanned but timed so that only parent/fragment pairs missing a certain mass will pass through); selection reaction monitoring/multiple reaction monitoring (quad one selects a mass, quad two fragments the selected mass, quad three selects the fragment). Multiple reaction monitoring (MRM) is commonly used for multiresidue pesticide analysis due to its sensitivity and selectivity.
3. **What are the main advantages of a QQQ over other mass spectrometric detectors?**
   QQQs are known for their sensitivity which makes it easy to detect dilute or low concentration analytes.
4. **What is backflushing and what purpose does it serve?**
   Back flushing is when the flow of the carrier gas in gas chromatography is reversed after the analytes have been detected. This can help keep the system clean as

residual compounds that had not left the column yet could be flushed out of the column and through the injection system.

6.6 Liquid Chromatography-Mass spectrometry

1. **How do we use aqueous and organic solvents as mobile phases for HPLC?**
   Isocratic mode or binary mode. The binary mode can ramp the percentage of organic phase to elute the compounds from low hydrophobic to high hydrophobic through the chromatography run.
2. **How does pH of the mobile phase affect the separation of compounds?**
   The pH of the mobile phase could change the hydrophobicity of a compound in solution. It may elute earlier or later than that under the initial pH.
3. **How do air bubbles change the results of HPLC?**
   Due to the air bubbles, the flow rate, and the ratio of aqueous-organic are changed, so that the retention time of a compound will shift. If it shifts out of the time window of detection, the peak(s) disappear.
4. **How does small leakage affect the results of HPLC?**
   The flow rate and the ratio of aqueous-organic are changed, so that the retention time of a compound will shift. If it shifts out of the time window of detection, the peak(s) disappear. In addition, the sensitivity will be lower.
5. **What factors can change the back pressure?**
   Leak, air bubble, wrong aqueous-organic ratio and malfunctional pumps.

6.6.1 HPLC Columns

1. **How does a C18 column separate the compounds in HPLC?**
   The balance of adsorption-desorption between a compound and C18 surface could be changed by the aqueous-organic ratio of the mobile phase. The hydrophilic compounds desorbed at lower ratio of organic, and the hydrophobic compounds desorbed at higher ratio of organic. Therefore, a compound is separated from a mixture solution.

6.7 Liquid Chromatograph with Triple Quadrupole Tandem Mass Spectrometry (LCMS or LC-MS/MS)

1. **Why it is important to stop the turbo pump before turning the power off?**
   The turbo pump works at low pressure, below atmospheric pressure. A mechanic pump can get the vacuum chamber to low vacuum. If both turbo and mechanic pump are turned off simultaneously, the atmospheric air pressure gets into vacuum chamber that would damage the turbo pump.
2. **How do we tell if an LC-MS/MS problem is due to HPLC or tandem mass spectrometry?**
   Do a manual injection after the HPLC column, if the signal (peak) is normal, the mass

spectrometry is normal, otherwise, the mass spectrometry is abnormal. After the problem of mass spectrometry resolved, check the system with manual injection again for HPLC portion.

3. **What are the trade-offs between the following: dwell time, number of ions monitored in an MRM Method and the number of data points in a chromatographic peak?**
   The data collection cycle is the sum of the dwell time of each ion in the monitoring window, when the number of ions is too high, the data collection cycle would be too long for the chromatography peak. Therefore, the trade-offs are to use shorter dwell time and shorter monitoring windows for higher efficiency.
4. **Why is the divert valve used between HPLC and tandem mass spectrometry?**
   The injection front and early elutes contains a lot of hydrophilic impurities from sample matrix make mass spectrometry dirty. A divert valve can switch the flow to the waste line to keep mass spectrometry clean. The same reason after the last compound elutes, the divert valve can divert the hydrophobic impurities, such as fat and oil, to waste.

6.8 Quantification

1. **Why are internal standards used? What is an ideal internal standard? How are internal standards used in quantitation?**
   Internal standards are compounds mixed into a sample that are used to normalize the response of the analytes across multiple injections. An ideal internal standard would have the same properties of the analyte (same polarity/retention time, response to the column, enhancement/suppression behavior) while remaining distinguishable from the actual analyte and not incurred in the samples. Isotopically labeled internal standards are often considered the best but can be expensive. In quantitation, the response ratio of the analyte to the internal standard is determined in the sample and compared to the response ratio in the standard solution (or against a multi-point response ratio calibration curve).
2. **Why is it important to review the chromatograms when performing quantitation?**
   While many software programs will allow you to review the calculated analyte concentrations without requiring the analyst to look at the chromatograms, the chromatograms can reveal whether the given result is reasonable. Evidence of interference, a saturated detector, or poor integration may not be evident without critically looking at the chromatograms.
3. **What are qualifier transitions and how do they differ from quantifier transitions?**
   Qualifier transitions are not usually used for quantitation of samples. They are intended to support the identification of the analyte. Qualifier/quantifier transition ratios should match the standard within a given percentage (SOP-000500 & WI-

000292) to determine if the analyte can be identified. While the quantifier transition is often the one that gives the largest response, qualifier ions may be more intense but prone to interference.

4. **How can issues such as split peaks or inadequate integrations be addressed?**
While most integration is automatically performed by the software to provide consistency across samples, sometimes these integration methods do not work for some matrix/analyte combinations. Manual integration is sometimes required to best capture the whole peak area. However, a better solution might be to alter the integration parameters to improve the problematic peak while still applying the same integration method to both the sample and standard.

6.5. Sample Analysis

A. Method Detection Limit (MDL)

1. **For what types of methods can an MDL be calculated? Do pesticides methods qualify?**
(Answer adapted from 40CFR136 Appendix B) MDLs can be calculated for well-defined analytical methods that are complete and specific. MDLs are not suitable for methods that give presence/absence or continuous distribution results. As pesticides analysis does provide distinct, numerical values as a result, the methods are usually suitable for MDL calculation.

2. **Give one way an MDL can be estimated prior to performing lab work.**
(Answer adapted from 40CFR136 Appendix B) (1) The mean determined concentration + 3 x SD of a set of method blanks; (2) The concentration value that gives an instrument SNR of 3 to 5; (3) 3 x SD of a set of spiked method blanks given as a concentration equivalent; (4) the region of the calibration where there is a change in the slope of the calibration; (5) previously determined MDL.

6.10 Sample Preparation and Compositing

1. **How would someone prepare a sample of 20 melons submitted to the laboratory?**
Remove and discard stems and composite whole melons using food processor. For smaller food processors equal portions of each melon may be used for compositing. (PAM I, 102- 2, 203-3)

2. **How would someone prepare a sample of six l-gallon cartons of milk?**
Composite by hand mixing equal portions from each carton. (PAM I, 102-4)

3. **How would someone prepare a sample of 20 lb of wheat?**
Mix sample thoroughly. Mill about 4 qts through 1-3 mm sieve. (PAM I, 102-3, 203-4)

4. **How would someone prepare a sample of slightly decomposed heads of lettuce?**
Very decomposed heads of lettuce? Cut out decomposed portion of lettuce heads

and composite remaining lettuce using food processor. Do not analyze very decomposed product. (PAM I, 102-2)

5. **What are the major considerations for a laboratory sample regarding the sample received by the laboratory and storage of reserve portions?** Sample integrity is to be maintained by storing the sample in locked cabinets or refrigerator/freezers. Composites are stored under conditions to preserve the residues and commodity as much as possible. (PAM I, 102-6)

6.11 Nonfatty Foods-Multiresidue Determination

1. **Briefly describe the extraction and cleanup principles involved in this assignment.**
   Acetonitrile is added to the sample to pull out pesticides from the relatively polar matrix. For dry products, water is also added to hydrate the dry product and improve extraction to the organic phase. Salts are added to push more compounds into the organic layer and to absorb some of the water (either added or from the matrix).
2. **What salts are used in the QuEChERS extraction and what function does each perform?**
   Sodium chloride and magnesium sulfate are used in the extraction. Sodium chloride drives more pesticide residues from the aqueous phase into the organic layer. Magnesium sulfate absorbs the water from the matrix to further encourage more pesticide residues to move into the acetonitrile. It also absorbs water that dissolves in acetonitrile.
3. **How does the extraction diverge for LC and GC extracts?**
   The evaluation of acetonitrile extracts by GC is not optimal and can cause poor results and eventual early column fouling. To improve GC analysis, the sample is diluted with ethyl acetate. An additional cleanup also removes matrix interferences, such as chlorophyll, that can impede GC quantitation. For LC extracts, acetonitrile is not a concern, and analysis can proceed directly after the salting out or an optional PSA based clean up can be applied.

6.12 Fatty Foods-Multiresidue Determination

1. **Briefly describe the extraction and cleanup principles involved in the assignment. How is the extraction different from the high moisture procedure performed?** High fat samples require additional assistance to bring non-polar analytes into the extraction solvent. In this case, the sample amount is reduced to limit adverse effects from the matrix on the extraction.
2. **What is the purpose of the additional water added to fatty samples?** Water helps to dilute interferences due to matrix on the extraction step. Among three liquid phases present in the extraction step, fat or oil, organic solvent and aqueous layer, the fats in these samples can retain non-polar analytes and reduce their extraction efficiency. Additional water can limit these effects.

6.14 Pesticide Residue Confirmation

1. **Where do you find listed the official tolerances for pesticide residues?**
   Official residue tolerances can be found in the 40 CFR 180.
2. **When the level of a residue exceeds an official tolerance, what type of analysis is performed?**
   How close must this agree with the original analysis? A check analysis must be performed and the quantification values from the original and the check should agree within 30% RPD. Per WI-000292, greater RPDs might be acceptable for residue levels near the LOQ.
3. **If there is no tolerance for a residue, what value must the residue level exceed?**
   The analytical level must exceed the LOQ or Limit of Quantitation.
4. **In the identification of residues, what is required to "Confirm" the identity of the residue?**
   Normally, there must be retention data on two different column types to confirm a residue. However, if a GC-mass spectrometer is used for the identification, only one column is required.
5. **When is recovery information required?**
   Recovery data is required when a residue that was not previously subjected to validation is to be reported for regulatory purposes.
6. **Is quantification always required on Original and Check analyses?** No, if there is no tolerance for the residue, then the check analysis can be confirmatory only, indicating that the residue has been confirmed in a second test portion of the commodity.