

**United States Department of Agriculture
Agricultural Marketing Service, Science & Technology
Pesticide Data Program**

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Title: Quantitation and Confirmation of Pesticide Residues		
Revision: 7	Replaces: 07/01/03	Effective: 07/01/04

1. Purpose:

To provide standard procedures for quantitating and confirming pesticide residues reported by laboratories participating in the USDA/AMS Pesticide Data Program (PDP).

2. Scope:

This standard operating procedure (SOP) shall be followed by all laboratories conducting residue studies for PDP, including support laboratories conducting stability or other types of studies that may impact the program.

3. Outline of Procedures:

- 5.1 Retention Time Criteria
- 5.2 Calibration Integrity
- 5.3 Quantitation Using Calibration Curves
- 5.4 Quantitation Using Single Point Comparisons
- 5.5 Data Confirmation Procedures
- 5.6 Quantitation of Pyrethroid Compounds
- 5.7 Subtraction of Incurred Residues

4. References:

- USDA/AMS PDP Quality Assurance (QA)/Technical Meeting, May 18-20, 2004
 - SOP PDP-DATA-02, Attachment 01, PDP Laboratory Information Form (LIF) Codes
 - USDA/AMS PDP Quality Assurance (QA)/Technical Meeting, April 9-11, 2002
 - USDA/AMS PDP Quality Assurance (QA)/Technical Meeting, February 21-22, 2001
 - Elizabeth Mishalanie, Enigma Analytical, May 9, 2000
 - Injector Reproducibility Study, Florida Department of Agriculture and Consumer Services, April 7, 2000
 - USDA/AMS PDP Quality Assurance Meeting, April 4-5, 2000
 - Mass Spectrometry (MS) Panel, April 2000
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- USDA/AMS PDP Quality Assurance Meeting, May 18-20, 1999
- 40 CFR 160.63, Maintenance and calibration of equipment
- 40 CFR Part 136 Appendix A

5. Specific Procedures to be Followed:

This SOP represents minimum PDP requirements and is presented as a general guideline. Each laboratory shall have written procedures that provide specific details concerning how the procedure has been implemented in that laboratory.

5.1 Retention Time Criteria

a. A residue in the sample is determined by satisfying the following criteria:

1. If an internal standard is used, the relative retention time (RRT) of the compound of interest to the internal standard within the reference standard and the RRT of the compound of interest to the internal standard within the sample shall be within 0.01.
2. If an external standard is used, the retention time (RT) of the compound of interest in the standard and the RT of the same compound in the sample shall be within 0.05 minutes (GC)/0.5 minutes (LC).

5.2 Calibration Integrity

Calibration integrity is defined as steady instrument response to a given amount of analyte over the duration of a sample run. Calibration integrity shall be determined by injecting standards at the beginning and end of a run and evaluating the variability in instrument response and any changes in retention time. Calibration integrity shall be calculated in terms of relative percent difference (RPD) using the following equation:

$$RPD = \frac{|X_1 - X_2|}{\frac{X_1 + X_2}{2}} \times 100,$$



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$$[(X_1 + X_2)/2]$$

where X_1 is the response of the first analytical standard injected and X_2 is the response of the second standard injected.

Changes greater than 20% relative percent difference (RPD) in response and 0.05 minutes (GC)/0.5 minutes (LC) in retention time indicate that intermediate standards are required to maintain calibration integrity. Each laboratory shall document exceptions in internal SOPs.¹ Each laboratory shall determine the number of intermediate standards required throughout the run to maintain calibration integrity. Injection order shall be determined by the individual laboratory.

5.3 Quantitation Using Calibration Curves

- a. Whenever possible, calibration curves shall be constructed using standards which bracket the expected range of residue concentrations. A suggested range is limit of quantitation to ten times the limit of quantitation. Second-order curves (i.e., quadratic) may be employed, providing that a sufficient number of points (i.e., minimum of five) is used to define the curve.
- b. Fitness of curve, whether first- or second-order, shall be demonstrated by one of the following accepted methods:
 1. correlation coefficient (where $R \geq 0.995/R^2 \geq 0.990$),
 2. percent relative standard deviation [%RSD (where $\%RSD \leq 20$)]:

$$\%RSD = [SD / (\text{avg. RF})] \times 100,$$

where SD is standard deviation:

¹ The laboratory may perform instrument-specific retention time studies to verify stipulation of different retention time window criteria than those specified in this SOP. It is expected that a generally accepted method of retention time window calculation be used and documented to establish these criteria.

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$$SD = \sqrt{\frac{\sum_{i=1}^n (RF_i - \overline{RF})^2}{n - 1}}$$

and RF is response factor, or the area or height of each standard divided by the concentration of that standard, or

3. percent difference of calculated vs. known standard concentration (where difference is within 15% and calculated as follows):

$$\text{Percent Difference} = (*C_1 - C_2^*/ C_1) \times 100,$$

where C_1 is the known concentration of the standard and C_2 is the concentration calculated using the calibration curve. For example:

Standard	Known Concentration (ug/mL)	Calculated Concentration from Curve (ug/mL)	% Difference
1	100	97	3
2	10	9.8	2
3	1	0.95	5
4	0.1	0.094	6
5	0.01	0.009	10

- c. During validation procedures the laboratory shall establish and document the methodology/parameters used. The laboratory shall specify in an internal SOP the method/parameter(s) used to demonstrate fitness of curve. Occasionally, determine the response of the detector at the limit of detection.
- d. Results obtained using a calibration curve shall lie within the range of the calibration curve. If results fall outside the calibration curve, the sample must be diluted, the calibration curve extended, or the procedures for single point comparisons followed. The procedure for extending the range of the

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calibration curve shall be documented in internal laboratory procedures. Data generated to support extension of the calibration curve shall be maintained and housed with the QAU.

5.4 Quantitation Using Single Point Comparisons

Quantitation using peak matching of a sample response to a single standard response is permitted if the sample response is within the established linear range. However, sample response shall fall within 30% of the standard response for samples greater than LOQ; if it does not, dilution of the sample or injection of a different standard concentration shall be required. This difference shall be calculated using the following equation:

$$[(X_{\text{standard}} - X_{\text{sample}}) \div (X_{\text{standard}})] \times 100,$$

where X_{standard} is the response of the standard and X_{sample} is the response of the sample.

5.5 Data Confirmation Procedures

All residues detected at concentrations that are equal to or greater than the established and verified LOD for a given analyte shall be confirmed. The method available for confirmation shall be capable of detecting the desired residue at a concentration that is equal to or less than the concentration quantitated by the first analytical method. All residues that cannot be confirmed by an acceptable confirmation method (see subsections 5.5.a through 5.5.d of this SOP) shall be reported as non-detects on the LIF. The confirmation method shall be reported on the LIF [refer to SOP PDP-DATA-02, Attachment 01, PDP Laboratory Information Form (LIF) Codes].

When more than one confirmation method has been utilized, the method with the higher level of confidence shall be entered in the confirmation method 1 field and the method with the next highest level of confidence shall be entered in the confirmation method 2 field. For example, a residue is confirmed using an alternate column and mass selective detector: the laboratory shall enter "M" in the confirmation method 1

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field and “D” in the confirmation method 2 field of the LIF. The decision regarding the level of confidence of a particular confirmation is left to the discretion of the Technical Program Manager. *[Note: the ident points (identification points) field of the LIF is an optional field that may be used to record the degree of confirmation.]*

a. Acceptable confirmation methods for GC analysis are:

1. Alternate detector (element specific/selective detectors, including various forms of mass spectrometry or atomic emission detection). Where possible, mass spectral confirmation is preferred. All applicable confirmation criteria for that detector must be met.
2. Mass spectrometer as an alternate detector. In some cases, confirmation criteria as specified in PDP-QC-06 and PDP-QC-09 may not be met, but sufficient information is available for confirmation by retention time and limited molecular data. The mass spectrometer may be utilized as an alternate detector in this instance if the following conditions are met:
 - a. The target ion, T1, and the primary qualifier ion, Q1, meet the ion ratio requirements as specified in SOPs PDP-QC-06 and PDP-QC-09.
 - b. T1, Q1, and the secondary qualifier ion, Q2, meet the three times noise requirement.
3. Alternate column, provided the alternate column changes the elution order or significantly changes (i.e., 2 or 3 peak widths) the retention time (RT) of the detected pesticides.

b. Acceptable confirmation methods for HPLC analysis are:

1. Alternate detector (element specific/selective detectors, including various forms of mass spectrometry or atomic emission detection).
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Where possible, mass spectral confirmation is preferred. All applicable confirmation criteria must be met.

2. Mass spectrometer as an alternate detector. In some cases, confirmation criteria may not be met, but sufficient information is available for confirmation. The mass spectrometer may be utilized as an alternate detector in this instance if the following conditions are met:
 - a. The target ion, T1, and the primary qualifier ion, Q1, meet the ion ratio requirements.
 - b. T1, Q1, and the secondary qualifier ion, Q2, meet the three times noise requirement.
 3. Alternate column, provided the alternate column changes the elution order or significantly changes (i.e., 2 or 3 peak widths) the RT of the detected pesticides.
 4. Alternate mobile phase, provided the alternate mobile phase changes the elution order or significantly changes (i.e., 2 or 3 peak widths) the RT of the detected pesticides.
 5. The acceptable confirmation method for HPLC using a diode array detector (DAD) is multiple signal detection for two characteristic wavelengths. During method development, the laboratory shall establish acceptance criteria for:
 - a. The peak ratio of the sample (peak height at wavelength 1 divided by peak height at wavelength 2) versus the peak ratio(s) of the standard(s).
 - b. The spectral match factor.
 - c. The peak purity factor.
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- c. Acceptable confirmation guidelines for mass spectrometry are specified in SOPs PDP-QC-06 and PDP-QC-09. These guidelines are for qualitative purposes only.

5.6 Quantitation of Pyrethroid Compounds

For pyrethroid compounds, the laboratory may base the LOD on the largest peak if a mass spectrometry system is used. If other systems are used, the laboratory must base the LOD on the smallest peak.

5.7 Subtraction of Incurred Residues

- a. For matrix spikes, the laboratory may subtract the incurred residue level from amounts recovered prior to calculating the percent recovery if the following conditions are met.
 - 1. Blank matrix cannot be obtained. The laboratory shall make every effort to obtain blank matrix such as purchasing organic produce, saving analyzed samples that are pesticide free, etc.
 - 2. The MPO Technical Director is notified that blank matrix is not available and concurs.
 - 3. The incurred residue level is less than two times the limit of quantitation
- b. The laboratory shall report blank subtracted spike recovery data using the code "S" and enter the amount subtracted into the comments field.



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6-16-04

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April 2004

PDP QA/Technical Meeting

- Added documentation procedures for extending the range of the calibration curve to Subsection 5.3.d
 - Added procedures for pyrethroid compound quantitation to Subsection 5.6
 - Added procedures for subtraction of incurred residues to Subsection 5.7
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