Washington State Department of Agriculture **Cannabis Program**

Cannabis Pesticide Residual AnalysisQC

1. Scope and Application

1.1. This method standard was adapted from the United States Department of Agriculture Pesticide Data Program.

1.2. These standards shall be followed by all analytical laboratories conducting pesticide residue analysis for cannabis and cannabis products in Washington state, including support laboratories conducting stability or other types of studies that may impact the program.

1.3. To provide a reference of cannabis pesticides, process controls, specification of WSDA commodity groupings, requirements for method validation and continuing quality control (QC) for Washington state cannabis testing.

2. Outline of Procedures

3.0 Specific Procedures
3.1 Required Compounds
3.2 Method Validation Background
3.3 General Method Validation Requirements
3.4 Method Validation Evaluation Guidelines
3.5 Method Validation Scenarios
3.6 Marker Pesticides
3.7 Process Control Compounds
3.8 Commodity Groupings
3.9 Establishment of LODs and LOQs
3.10 Verification of LODs/LOQs
3.11 Changing LODs
3.12 Determination of Method Range
3.13 Precision and Accuracy Data Collection
3.14 Method Evaluation Reporting
3.15 Method Validation Evaluation by WSDA
3.16 Blanks and Spikes Required Per Set and Continuing QC
3.17 Criteria for Method Validation and Continuing QC
3.18 Proficiency Testing
3.19 Measurement Uncertainty
4.0 References
5.0 Acknowledgements

3. Specific Procedures

3.1. Required Compounds

3.1.1. Refer to applicable commodity/compound-specific memoranda for commodity specific testing profiles.

3.1.2. Priority Levels

3.1.2.1 Each analyte of interest for each assigned commodity shall be designated with a priority level.

3.1.2.2 In the various commodity-specific memoranda compounds identified as Priority 1 compounds are the most critical and those identified as Priority 4 are the least critical. The priority level is a combination of data needs and expected feasibility of current methods to recover a given compound. General priority levels are assigned according to the following protocol:

3.1.2.2.1 Priority 1 compounds are selected in rules by the Washington State Liquor and Cannabis Board and the Washington State Department of Health.

3.1.2.2.2 Priority 2 compounds include other multiresidue-amenable compounds with a current tolerance for the given commodity that are highly important because they also have upcoming reviews scheduled or have been identified by a stakeholder as an important data need. Cyphenothrin, imiprothrin, and tetramethrin are also included as priority level 2 compounds for all commodities. Additionally, chemicals used in other countries may be included as Priority 2 compounds, dependent upon their anticipated method behavior.

3.1.2.2.3 Priority 3 compounds include other analytes with tolerances (including food handling establishment tolerances) or ALs (e.g., environmental contaminants/extraneous residues – aldrin, BHC, chlordane, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene, lindane, mirex, and oxychlordane) for the given commodity and are routinely analyzed by multiresidue methods. Priority 3 compounds may also include chemicals used in other countries, dependent upon their anticipated method behavior.

3.1.2.2.4 Priority 4 compounds include pesticides that have current tolerances, but likely require single analyte methods (e.g., glyphosate/AMPA, paraquat/diquat, EBDCs). Priority 4 compounds may also include chemicals used in other countries, dependent upon their anticipated method behavior.

3.1.2.3 All methods must test priority 1 compounds. WSDA may designate other compounds as priority 2,3, and 4 compounds.

3.1.2.4 In some cases, WSDA will authorize the development of new methods to detect certain compounds.

3.1.3 Ordering Analytical Standards

3.1.3.1 Procurement of standards from all sources must meet the following minimum requirements:

Availability of a current and valid “Certificate of Analysis” (CoA) (as a minimum requirement the certification shall identify the substance, its purity, and the production lot), traceability, and current expiration date. All standards require a certificate of analysis.

3.1.3.1.1 Certified standards compliant with ISO Guide 34 should be used when available.

3.1.3.1.2 An exemption for CoA and current expiration date is allowed for extraneous environmental contaminants that are covered by FDA Action Levels and compounds that have been revoked and no longer have existing U.S. registrations. Extraneous environmental contaminants include aldrin, BHC, chlordane, DDD, DDE, DDT, dieldrin, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene, lindane, mirex, and oxychlordane. Examples of revoked compounds that no longer have existing U.S. registrations include parathion ethyl, chlorfenvinphos, and fenchlorphos.

3.1.3.1.3 For all other analytical standards, in some cases, a current and valid CoA may not accompany the analytical standard. In this case, the laboratory shall contact the vendor to determine if one is available.

3.1.4 Receipt of Analytical Standards

Custody of a standard begins when the standard is received in the laboratory. Each standard shall be given unique identifier that differentiates the standard from neat material to final dilutions. Receipt of standards shall be documented, and each standard shall be traceable. Records shall include name, unique identifier, purity, lot number, date received, and expiration date.

3.1.5 Storage of Analytical Standards

3.1.5.1 Neat standards shall be kept in a separate standards freezer, preferably at approximately -20°C or lower unless degradation occurs at such temperatures. In these cases, neat standards shall be stored at the manufacturer’s recommended temperature.

3.1.5.2 Stock standards and dilutions including mixed standards shall be kept in refrigerators or freezers separate from those used for samples to avoid contamination. Stock standards and dilutions shall be stored in teflon-lined, screw-capped, glass bottles or sealed glass ampules.

3.1.5.3 Access to the freezers and refrigerators shall be controlled and standards usage documented through the use of appropriate records (e.g., log books). These records shall contain at a minimum: standard name and/or unique code, date and time removed, initials of person removing standard, date and time returned, initials of person returning standard.

3.1.5.4 Refrigerator and freezer temperatures shall be checked either by taking readings each working day, or by automatic temperature recording devices that are checked each day of operation.

3.1.5.5 When a neat standard is removed from freezer storage, it is recommended the standard be stored in a desiccator while it is brought to room temperature to minimize the potential for hydrolysis.

3.1.6 Preparation of Stock Standard Solutions

3.1.6.1 Stock standard solutions should be prepared in a separate standard preparation area to avoid contamination of samples with pesticide standards. A separate standard preparation area is not required if there are appropriate cleaning procedures and controls to ensure against cross contamination.

3.1.6.2 Each stock standard shall be given a unique identifying code and shall be labeled (or labeled by reference) with a minimum of: pesticide name, concentration, solvent, date of preparation, initials of preparer, and expiration date of solution. Written SOPs for stock standard preparation shall include the method for preparing standards, calculations used in standard preparation, documentation that provides for standard traceability and safety guidelines.

3.1.7 Preparation of Intermediate Dilutions

3.1.7.1 Intermediate dilutions, including mixed standards, shall be prepared in a separate standard preparation area. A separate standard preparation area is not required if there are appropriate cleaning procedures and controls to ensure against cross contamination.

3.1.7.2 Each standard shall be given a unique identifying code and shall be labeled (or labeled by reference) with pesticide name, concentration, solvent, date of preparation, initials of preparer, and expiration date of solutions. Written SOPs shall include the method for standard preparation and documentation that provides for standard traceability.

3.1.8 Independent Calibration Verification (ICV)

A solution of pesticide analytes prepared from a second source that is independent of the source used to prepare the calibration standards. It is used to verify the calibration.

3.1.8.1 New stock solutions that are prepared from neat pesticides currently used in the laboratory shall be compared to the old stock solution. The two standards must match within 15% RPD. If the two standards do not match, the problem must be identified and solved before the standard is used for quantitation. A suggested approach is to make new dilutions of both the old and new standards to check for dilution errors. If no dilution errors are found, a second stock dilution should be made to determine whether an error was made in the original preparation from neat material.

If these two stocks match, then the standard may be used. If they do not match, a third stock solution should be made. Whenever possible, duplicate injections shall be used.

3.1.8.2 Documentation of the standard checking process shall be kept through appropriate records (i.e. logs). Chromatograms of all standards shall be kept indicating the standard comparisons of old and new standards and the calculated difference.

3.1.9 Working Dilutions/Mixed Standards

3.1.9.1 Working dilutions and mixed standards shall be checked to ensure integrity of the solutions. These solutions should be made as frequently as necessary to ensure that concentrations do not change and/or individual pesticides do not degrade. Each laboratory shall determine the frequency of remaking dilutions/mixed standards. Documentation supporting this decision shall be maintained. A suggested guideline is six months for stock mixed standards and one month for working dilutions. Some pesticides may require more frequent dilution from the stock.

3.1.9.2 An archive file of all old mixed standards shall be kept and the dates the standards were used shall be indicated. The archive file shall be maintained a minimum of five years.

3.1.9.3 All working/mixed standards shall be identified by a unique and traceable code. Working/mixed standard records shall contain a minimum of pesticide name, solvent, date of preparation, expiration date, and preparer.

3.1.10 Detector Profiles

Standard retention time and response shall be characterized by analysis on the detectors used in each laboratory. These include but are not limited to: GC-ECD, GC-FPD, GC-ELCD, GC- XSD, GC-MSD, GC-ITD, LC-MS, and tandem MS. Libraries of all standards shall be developed for confirmatory instruments (GC-MS and LC-MS systems).

3.1.11 Disposal of Analytical Standards

Each laboratory shall establish the proper procedures for disposal (e.g., disposal by a licensed contractor) of expired analytical standards (both neat standards and dilutions). Disposal shall be in accordance with the laboratory's standard operating procedures and shall be documented.

3.2 Method Validation Background

3.2.1 All pesticide compounds designated as required by the LCB and DOH are marker compounds. Priority 1 compounds and marker compounds shall be the same list of compounds.

3.2.2 This method evaluation framework makes the following assumptions:

3.2.2.1 Commodities are grouped in such a way that assessment of method performance in one commodity in the group can be extended to apply to all commodities in the group.

3.2.2.2 LOD is specific to a pesticide-commodity pair and must be evaluated for every pesticide-commodity pair.

3.2.2.3 Although a method may be extended to other commodities and pesticides, a minimum amount of LOD verification and recovery data must be obtained to confirm this assumption.

3.2.3 This SOP details various scenarios and their corresponding method validation requirements.

3.2.4 When problems occur, such as instrument reproducibility and/or linearity, an investigation of causes shall be conducted.

3.3 General Method Validation Requirements

3.3.1 Methods selected for use by accredited laboratories, and significant changes to approved methods, are subject to prior approval by WSDA.

3.3.2 The laboratory shall complete all required method validation modules, with the exception of precision and accuracy data collection (extracted, analyzed, and reviewed) prior to the extraction of any routine analytical sample sets.

3.3.3 An extraction/detection system includes the whole method: extraction, clean-up, chromatography, and analytical technique.

3.4 Method Validation Evaluation Guidelines

3.4.1 The following possible scenarios shall be followed for validation of new methods or changes/additions to existing methods:

3.4.1.1 Implementing a new method (3.5.1)

3.4.1.2 Changing an analytical method (3.5.2)

3.4.1.2.1 Extraction (3.5.2.1)
3.4.1.2.2 Post-extraction/pre-instrumentation (3.5.2.2)
3.4.1.2.3 Instrumentation - new Limit of Detection (LOD) (3.5.2.3)
3.4.1.2.4 Minor Modifications (3.5.2.4)

3.4.1.3 Adding a new commodity grouping (3.5.3)

3.4.1.4 Adding a raw agricultural commodity or a processed commodity to an existing commodity group. (3.5.4)

3.4.1.5 Adding pesticides related to marker pesticide groups to an existing commodity group (3.5.5)

3.4.1.6 Adding a new pesticide that is not related to marker pesticide groups to an existing commodity group. (3.5.6)

3.4.2 Evaluation takes place through the performance of method evaluation modules. These modules are chosen to meet the requirements of each scenario. The modules are:

- Establishment of LODs and Limits of Quantitation (LOQs) (3.9)
- Verification of LODs/LOQs (3.10)
- Determination of Method Range (from 1xLOQ to 10xLOQ) (3.12)
- Precision and Accuracy Data Collection at 2xLOQ (3.13)
- Method Evaluation Reporting (3.14)

3.4.3 Section 3.5 of this SOP lists each scenario and the modules that must be performed in that scenario. Sections 3.9 through 3.14 outline the detailed procedures to be followed for each module.

3.5 Method Validation Scenarios

The WSDA will determine which scenario described in the following subsections applies for the analytes/commodities/methods pairings.

3.5.1 New method implementation – Proceed with:

- Establishment of LODs and (LOQs) (3.9)
- Verification of LODs/LOQs for all compounds (3.10)
- Determination of Method Range for marker compounds (3.12)
- Precision and Accuracy Data Collection for all compounds (3.13)
- Method Evaluation Reporting (3.14)

3.5.2 Method Changes

3.5.2.1 Major Extraction Change - Examples would be using a different solvent, solid phase extraction (SPE) sorbent bed, or a new technique. Proceed with:

- Establishment of LODs and (LOQs) (3.9)
- Verification of LODs/LOQs for all compounds (3.10)
- Determination of Method Range for marker compounds (3.12)
- Precision and Accuracy Data Collection for all compounds (3.13)
- Method Evaluation Reporting (3.14)

3.5.2.2 Major changes in post-extraction/pre-instrumentation procedures (cleanup) - Proceed with:

- Verification of LODs/LOQs for all compounds (3.10)
- Determination of Method Range for marker compounds (3.12)
- Precision and Accuracy Data Collection for all compounds (3.13)
- Method Evaluation Reporting (3.14)

3.5.2.3 Instrumentation Changes - The WSDA will determine if the instrument change warrants completion of the following sections. The WSDA shall be notified of all instrument changes. This is dependent upon the extent of modification.

For new LOD - Proceed with:

- Establishment of LODs/LOQs for all compounds (3.9)
- Verification of LODs/LOQs for all compounds (3.10)
- Method Evaluation Reporting (3.14)

The laboratory shall use best professional judgment to determine if Precision and Accuracy Data Collection (subsection 3.13) is necessary.

3.5.2.4 Minor modifications of existing method - The WSDA will determine which portions of the following sections will be completed. The WSDA shall be notified of all modifications to existing methods. *This is dependent upon the extent of modification.*

- Establishment of LODs and LOQs of affected analytes (3.9)
- Verification of LODs/LOQs of affected analytes (3.10)
- Determination of Method Range of affected markers (3.12)
- Precision and Accuracy Data Collection of affected analytes (3.13)
- Method Evaluation Reporting (3.14)

3.5.3 Adding a new commodity group (see Section 3.8) Proceed with:

- Verification of established LODs/LOQs for all required pesticides in the new commodity (3.10)
- Determination of Method Range for the marker pesticides (3.12)
- Precision and Accuracy Data Collection for all required analytes (3.13)
- Method Evaluation Reporting (3.14)

3.5.4 Adding a cannabis product to an existing commodity group (see Section 3.8) Proceed with:

- Verification of established LODs/LOQs for all required pesticides (3.10)
- Precision and Accuracy Data Collection (2 points) for all required pesticides (3.13)
- Method Evaluation Reporting (3.14)

The laboratory shall use best professional judgment to determine if additional validation is necessary based on matrix behavior.

3.5.5 Adding pesticides related to the marker pesticide groups to an existing commodity group (see Section 3.8) – Proceed with:

- Establishment of LODs and LOQs for each pesticide added (3.9)
- Verification of LODs/LOQs for each pesticide added (3.10)
- Precision and Accuracy Data Collection for each pesticide added (3.13)
- Method Evaluation Reporting (3.14)

3.5.6 Adding pesticides that are not related to the marker pesticide groups to an existing commodity group (see Section 3.8): (For example, the addition of imidacloprid analyzed by the same multiresidue procedure. The new pesticide may then become a marker pesticide for similar pesticides that are later added.) - Proceed with:

- Establishment of LODs and LOQs for each pesticide added (3.9)
- Verification of LODs/LOQs for each pesticide added (3.10)
- Determination of Method Range for compound(s) that is/are to become marker(s) (3.12)
- Precision and Accuracy Data Collection for each pesticide added (3.13)
- Method Evaluation Reporting (3.14)

3.6 Marker Pesticides

3.6.1 Marker pesticide compounds are the required pesticides in lists established and maintained by the LCB and DOH.

3.6.1.1 Compounds are placed into marker groups based on a combination of analyte chemistry and method performance behavior when requested by a laboratory. Initial compound designations are made by WSDA, with applicable analytical laboratory input based on known method behavior, if those data are available. For new compounds, behavior data may not be available.

3.6.2 Multi-residue Screening

3.6.2.1 The laboratory must spike all compounds for each commodity group.

3.6.2.2 Upon WSDA approval, or if directed by WSDA, certified laboratories may employ the following: For laboratories using marker groups, each laboratory shall select at least one compound from each applicable group to serve as a marker pesticide. Applicable groups are those that contain at least one compound analyzed by that laboratory for that commodity. For each applicable group, a marker pesticide shall be included for each extraction/detection system used to analyze that group.

3.6.2.3 Upon WSDA approval, or if directed by WSDA, certified laboratories may employ the following: For laboratories rotating spike mixtures between analytical sets, each laboratory shall ensure that each extraction/detection system is adequately represented within each set.

3.6.2.4 Upon WSDA approval, or if directed by WSDA, certified laboratories may employ the following: For laboratories analyzing multiple commodities, a single list of marker compounds may be specified to represent all commodities. The lists of required compounds for commodities analyzed should be combined and at least one compound from each applicable group chosen to serve as a marker compound.

3.6.3 Upon WSDA approval, or if directed by WSDA, certified laboratories may employ the following: Selected/single analyte residue studies utilize the selected analyte as the marker pesticide.

3.6.4 Upon WSDA approval, or if directed by WSDA, certified laboratories may employ the following: "Marginal Performing Analytes" are analytes that do not meet linearity, calibration integrity, ion ratio, recovery (individual or mean), or precision and accuracy criteria during method validation or continuing quality control (QC). Marginal performing analytes are determined in conjunction with WSDA.

3.7 Process Control Compounds

Samples analyzed by each extraction/detection system shall include the analysis of a process control compound. More than one process control may be required. The laboratory shall make every effort to choose a compound that is not expected to be an incurred residue. This includes both internal standards and surrogate spikes. Either surrogate(s) or internal standard(s) must be used, but both are not required at the same time.

3.8 Commodity Groupings

Group 1:

- High THC Flower
- High CBD Flower
- High THC/ High CBD Flower (Hybrid)

"High" is established as levels ≥ 10%.

Group 2:

- Hydrocarbon and CO_2
  - Butane
  - Propane
  - Pentane
  - Heptane
  - CO_2

Group 3:

- Non-solvent Extracts
  - Kief
  - Hash
  - Rosin

Group 4:

- Infused Oils
    - Butter
    - Medium Chain Triglycerides

Group 5:

- Food Grade and Ethanol
    - Glycerin
    - Propylene Glycol
    - Ethanol

Group 6:

- High Purity
    - Distillates
    - Isolates

3.8.1 Based on their experience with a commodity, laboratories may request changes to the assigned commodity groupings from the WSDA.

3.9 Establishment of LODs and LOQs

3.9.1 Method Noise

3.9.1.1 Method noise is the combination of instrument noise and the matrix noise contributions.

3.9.1.2 Method noise determination must be completed for all required analytes.

3.9.1.3 Method noise will be determined utilizing instruments and operating conditions, which are routinely used for the analysis of samples. Noise for the LOD and LOQ calculations will be determined by examining chromatograms of the blank commodity in the chromatographic time segment of the pesticides of interest.

3.9.2 Establishment of LOD

3.9.2.1 LOD may be estimated by whatever means the laboratory chooses to employ, but the response shall be at least 3x signal to noise.

For MS systems, ions used for quantitation and for qualitative analysis/confirmation shall meet the 3x signal to noise requirement.

For example: 1) take two equal portions from the same matrix blank extract; 2) spike one aliquot with a known amount of the analyte of interest; 3) inject both aliquots under the same conditions; 4) magnify the baseline of the unfortified blank at the analyte retention time window of interest to obtain the instrument response for the tallest (height) or the broadest (area) noise; and 5) convert the response into concentration (ppm, ppb, or ppt) from the known concentration of the spiked extract.

Compare the two concentrations (blank vs. spiked) to estimate the LOD.

3.9.2.2 LODs may be established at a level greater than 3x noise.

3.9.2.3 In addition to signal-to-noise considerations, LODs estimated for zero noise instruments (e.g. triple quadrupoles) may also include consideration of replication injection data (e.g. injecting an LOD standard 10x).

3.9.2.4 The reported LOD shall be the highest value obtained using the validated method. For instance, for dual column systems, the confirmatory column LOD must be AT LEAST that of the primary/quantitative column.

3.9.2.5 For multi-peak compounds, such as many of the pyrethroids, the laboratory may base the LOD on the largest peak if a mass spectrometry system is used for both quantitation and confirmation. If other systems are used for quantitation, the laboratory may base the LOD on the larger peak if the smaller peak is <20% of the total response.

3.9.2.6 LOD is method dependent and shall be experimentally verified in matrix as detailed in Section 3.9.1.

3.9.3 Establishment of LOQ

3.9.3.1 LOQ will be calculated/determined for each analyte in each commodity tested following the establishment of LOD.

3.9.3.2 For all detection systems other than mass spectrometry, LOQ will be established by multiplying the response of method noise level by at least ten and then converting the total response into concentration (i.e., ppm, ppb, or ppt), or by multiplying the LOD by no less than ten/thirds (10/3) if the LOD is established above 3x method noise.

3.9.3.3 For mass spectrometric systems, ions to be used for qualitative analysis/confirmation shall be at least 3x signal to method noise. Ions to be used for quantitation shall be at least 10x signal to method noise.

3.9.3.3.1 In order to maximize the number of compounds screened by MS systems while maximizing the number of scans per second and dwell times, it may be desirable to perform the initial identification and quantitation using fewer than three ions for some or all of the compounds. Presumptive-positive samples shall be re-injected or data reprocessed to meet all MS confirmation criteria.

3.9.3.4 The reported LOQ shall be the highest value obtained using the validated method.

3.10 Verification of LODs/LOQs

3.10.1 During method validation, all calculated or established LODs must be verified by fortifying duplicate blank commodities at approximately the LOD level and subjecting them to the analytical method for each extraction/detection system used in the analysis of cannabis samples. In the instance where the LOD=LOQ this verification suffices for the LOD and LOQ. If method range is performed (see subsection 3.12) for verification of LOQ then section 3.10 is not required.

3.10.2 Verification consists of the observation of detectable peaks in the chromatogram at 3x the current noise level (run within the last three months). Variability is expected to be high. Therefore, recoveries can be reported as present or not present. If detectable peaks are not observed, the LOD must be re-estimated and the verification repeated.

3.10.3 Prepare summary form(s) of the acquired data for all systems and all columns used for analysis and/or confirmation.

3.11 Changing LODs

3.11.1 LODs may be raised for analytes in an individual sample set at the discretion of the WSDA.

3.11.2 LODs may not be lowered without verification subject to the analytical method, WSDA review and approval.

3.12 Determination of Method Range

3.12.1 During method validation, samples fortified with marker compounds (only marker compounds are required, however, other compounds may be used in addition to the markers, if desired) are to be run through the entire analytical method on the primary analytical system. If more than one type of chromatography system (e.g., GC versus LC) and/or detector system (e.g., FPD versus MSD) combinations are to be used for quantification, they must be likewise evaluated.

3.12.2 Fortify samples in triplicate at approximately 1xLOQ, 5xLOQ, and 10xLOQ for each marker or compound being validated. Process these fortified samples through the entire analytical method. A reagent and matrix blank shall be subjected to the analytical method along with the fortified samples.

3.12.3 For each data point, calculate the Percent Recovery compared to known standards to three significant figures if greater than 100% or to two significant figures if less than 100%.

3.12.4 Calculate the mean Percent Recovery (%R) and Coefficient of Variation (%CV) for each level.

3.12.5 Prepare summary form(s) of the acquired data by analyte, level, and commodity group.

3.12.6 Method Range Extension

If more than 20 findings per life of the commodity for a particular analyte/commodity pair exceed the highest validated spiking level, then in order to verify the ability of the method to extract the analyte at the higher level, the laboratory shall fortify at least one spike at or above the level of the highest finding. Reagent and matrix blanks shall accompany these spikes. If the laboratory control sample recoveries do not meet QC criteria any affected findings shall be coded (or recoded) as estimates. Marker pesticide spikes may be used to represent other compounds in that group. Method range extension for a given commodity can represent another commodity in that group. Laboratories may perform the range extension at various times:

3.12.6.1 Preemptively during initial validation (based on intelligence or experience with the commodity),

3.12.6.2 In subsequent batches following the high finding,

3.12.6.3 Periodically (e.g. annually) to conserve resources, or

3.12.6.4 Internal blind check samples may be used for this purpose.

3.12.6.5 Method range extension results should be reported to WSDA following QA review.

3.13 Precision and Accuracy Data Collection

3.13.1 The precision and accuracy data collection shall be compiled from the commodity groupings as specified by WSDA. Each marker, single analysis, new or other required analyte shall be spiked at 2xLOQ and evaluated using a minimum of seven data points, with at least two points from each commodity in the group analyzed in a particular laboratory.

3.13.2 The required data points shall be obtained from:

3.13.2.1 2xLOQ data points completed after Determination of Method Range

and/or

3.13.2.2 Data points from laboratory control sample analyzed concurrently with samples.

These two options provide slightly different data. The second option is preferable since it provides information about the repeatability of the method over time. The first option is permitted when running concurrent spikes would make the size of sample sets unmanageable.

3.13.3 For each data point, calculate the Percent Recovery compared to known standards to three significant figures if greater than 100% or to two significant figures if less than 100%.

3.13.4 Calculate the mean Percent Recovery (%R) and Coefficient of Variation (%CV) for each pesticide using the seven data points.

3.13.5 Prepare summary form(s) of the acquired data.

3.14 Method Evaluation Reporting

3.14.1 The methodology, method evaluation records, summary form(s), chromatograms, and any other supporting data generated during method evaluation shall be maintained by the laboratory.

3.14.2 Local Approval

3.14.2.1 Any request for and written modification of an approved analytical method shall be reviewed and approved by the QA Officer and Scientific Director.

3.14.2.2 All validation documentation shall be reviewed and approved by the QA Officer and Scientific Director.

3.14.3 Letter of Intent

3.14.3.1 Once the Verification of LODs and LOQs and Determination of Method Range has been completed, reviewed, and approved by the Scientific Director and QA Officer, a Letter of Intent shall be submitted to the WSDA stating that these modules have been completed, reviewed, and approved and will be submitted at a later date with the Precision and Accuracy Data.

3.14.3.2 This letter shall also include a list of commodity(ies) and analyte(s) with their LOD(s) that the laboratory intends to analyze and shall be submitted within 90 days of the applicable commodity entering the program.

3.14.3.3 The Letter of Intent is not required if all required method validation data will be/is submitted within 90 days of the commodity entering the program.

3.14.3.4 WSDA will perform a brief preliminary review and upon laboratory request, will issue a provisional letter allowing the laboratory to transmit data to their laboratory liaison for review while the full method validation package undergoes a multi-level review by WSDA. Data may be changed, in consultation with the lab, based on the results from the full method validation package review.

3.14.4 Upon conclusion of the Precision and Accuracy Data Collection module, summary form(s) of validation documentation, and a brief narrative shall be sent by email to the WSDA with a cover memo detailing the submission stating which version of the QC SOP was followed to determine the scenario(s) and module(s) that the submission is intended to represent.

3.14.5 A narrative accompanying the validation documentation shall include the following.

3.14.5.1 Description of the method.

3.14.5.2 Identification of any data that is only intended to be used for confirmation. Otherwise, WSDA will evaluate the data as if quantitation will be performed on the instrument/analyte combination.

3.14.5.3 Requests for designation of any analytes as Marginal Performing Analytes - if WSDA agrees to consider any analytes as Marginal Performing Analytes, that designation will be documented in the provisional letter.

3.14.5.4 Identification of previous method validation data used. The laboratory shall be responsible for clearly identifying the data used and the rationale for their use. For example, if a previously validated commodity returns and the laboratory has not made any method changes and will be using the same instrumentation, the laboratory shall submit a letter to WSDA explaining how the previous validation data will be used.

Example narrative for a data package:

Enclosed is the complete method validation summary of all compounds we are screening for in commodity “y” to support the addition of the commodity to the 2020 WSDA CLASP program. The method validation was performed according to the requirements of QC SOP version X. The specific scenario used in validation was 3.5.1, New Method Implementation. Required modules included establishment and verification of LODs and LOQs, determination of method range, precision and accuracy data collection, method evaluation reporting for GC/MSD, GC/FPD, GC/XSD, and LC/MS/MS instrumentation. For compound “a”, GC/FPD is the primary detection system and LC/MS/MS data is intended for confirmation purposes only. Due to problems with recovery, the following analytes should be considered Marginal Performing Analytes and if it is agreed, will be coded as such in reporting: compound “e”, compound “f”, and compound “g”. If there are questions about this submission please contact: XXXXXX. All references to this submission should use QA# ###- ####.

Example narrative for a previously validated returning commodity with no method, analyte, or instrumentation changes:

In 2020, commodity “y” returned to the 2020 CLASP program. This commodity was previously validated in 2017 and there have been no changes to the method, target analytes, and instrumentation since then. Therefore, the 2017 validation data submitted on Month, Day, Year, is still applicable. If there are questions about this submission please contact: XXXXXX. All references to this submission should use QA# ###-####.

An example format for the submission follows:

Title

Summary to include purpose, results, data anomalies. Methods

Sample Preparation (example):

- 50g homogenized sample extracted with 100 ml ACN by gently mixing
- 5ml extract purified by a C-18 SPE cartridge, eluted with MeOH, and concentrated to 5ml
- 1 ml eluate further purified by florisil SPE and eluted with 5 ml 50:50 hexane/acetone
- Eluate dried down to 0.5 ml, re-suspended in acetone, and filtered
- Derivatization accomplished by reaction with dansyl chloride.

Analysis (example):

- Instrument GC/HPLC/detector
- Column (DB-)
- Post-column derivatization (where applicable).

Method Validation Evaluation by WSDA

3.14.6 Letter of Intent

3.14.6.1 Letters of Intent shall be tracked and maintained in centralized files by the WSDA.

3.14.6.2 The WSDA laboratory liaison assigned to that facility submitting a Letter of Intent shall review the letter and verify the submitted LOD/LOQ values against electronically submitted data (upon availability) and upon laboratory request issue a provisional letter (see Section 3.14.3).

3.14.7 Method Validation Data Packages

3.14.7.1 After receipt by the WSDA, method validation data packages undergo a multi-tiered review.

3.14.7.2 The method validation package is reviewed to ascertain the presence and completeness of data submitted for method validation and to determine whether these data adhere to WSDA criteria and are to be considered validated.

3.14.7.3 Data that do not meet WSDA criteria for linearity, calibration integrity, ion ratios, individual or mean recovery (70-130%) or reproducibility (%CV values within the expected Horwitz intralaboratory values) shall be reported to the CLASP program manager. WSDA and the laboratory shall use scientific judgment to determine whether the compound shall be considered validated, designated as a Marginal Performing Analyte or designated as unvalidated for that pesticide/commodity pair.

3.14.7.4 Once the WSDA review of the method validation package has been completed, the laboratory Scientific Director and QA Officer will receive a provisional letter that identifies the status of the instrument/detector results for the commodity/analyte pairing (e.g., validated, not validated, Marginal Performing Analyte, incomplete). If the data are deemed incomplete by WSDA, the provisional letter will identify the deficiency and include a request for the remaining data (e.g., monitoring of daily matrix fortifications or addition of a spike compound with the same functional group to the fortification profile).

3.14.7.5 Once a compound is designated as a Marginal Performing Analyte, that designation shall not be changed unless approved by the WSDA.

3.15 Blanks and Spikes Required Per Set and Continuing QC

3.15.1 Sample Set

A sample set is a group of samples, which are spiked individually with the designated process control(s), extracted with the required QC samples, and analyzed with the applicable required QC samples. Each set shall not exceed 20 samples. Required QC samples per set consist of a reagent blank, matrix blank, and laboratory control sample(s).

3.15.2 Reagent Blank

A reagent blank is intended to demonstrate glassware cleanliness and total system integrity through the use of all reagents during the extraction procedure. It shall be prepared by subjecting an amount of distilled water equivalent to that contained in an average sample to the entire analytical process. For consistency in the preparation of the reagent blank, it shall be assumed that an “average” cannabis sample contains 10% water. If contamination or interferences in the retention time window of the pesticide of interest is present in excess of the calculated LOQ, appropriate action must be taken and documented.

3.15.3 Matrix Blank

A matrix blank is intended to demonstrate the behavior of a substrate within an analytical system. Ideally, a matrix blank should be void of any compounds of interest. A matrix blank may be a previously characterized sample of the same commodity. If a suitable sample is not available, a portion of one of the samples may be randomly selected and used as a matrix blank. If an incurred residue is found in the matrix blank, which has been chosen from the sample set, determine if the same residue is incurred in the actual sample and is not present in other samples in the same set. If this condition cannot be met, appropriate action must be taken, such as reviewing reagent blank information.

3.15.4 Laboratory Control Sample

A laboratory control sample is intended to reflect the behavior of a chemical in a substrate within an analytical system. The laboratory control sample indicates the behavior of the chemical for the entire sample set. Analysis of a laboratory control sample provides valuable information on matrix interference effects as a result of the co-eluted matrix components, affecting the accuracy or detection capability for the analytes of interest.

3.15.4.1 A second portion of the same material used for the matrix blank shall be used for the laboratory control sample(s). Laboratories may design their QC spiking schemes to meet their needs. A laboratory may choose to use marker groups as defined in Section 3.6 of this method standard, rotate spike mixtures between analytical sets, or spike all compounds analyzed, as long as each extraction/detection system is adequately represented within each set and the minimum requirement of all compounds reported by the laboratory to be spiked at least quarterly in each commodity, is met.

3.15.4.2 The spike shall be added prior to extraction at approximately 5x LOQ (or less). Additional spikes may be added to satisfy the quarterly spiking of each commodity with all reported compounds, as part of a validation study, or to familiarize a laboratory with pesticides that have not been previously analyzed. More than one laboratory control sample shall be required if necessary for all spiked compounds to be separated during the chromatographic process.

3.15.4.3 Incurred residue levels may be subtracted from the spike recovered prior to calculating the percent recovery if the conditions specified in Pesticide Data Analysis are met.

3.15.5 Process Control Spikes

A process control spike is intended to assure the integrity of a particular sample within an analytical system.

3.15.5.1 Each sample set component, except the reagent and matrix blanks, shall be spiked with a process control at approximately 5x the Limit of Quantitation (LOQ) prior to the extraction step of the analytical procedure. However, if the intent of the process control is to monitor the percent recovery of a clean-up step, or of a derivatization, then the process control shall be added to the extract before the clean-up or derivatization step.

3.15.5.2 The laboratory shall make an effort to choose a compound that is not expected to be an incurred residue. The value reported as "percent recovery" may be the original, re- injected, re-aliquoted, or re-extracted (from homogenate) determination value [either value from primary detection system or averaged value (e.g., dual column results averaged)].

3.16 Criteria for Method Validation and Continuing QC

3.16.1 Method Validation Criteria

3.16.1.1 WSDA criteria for percent recovery for determination of method range and precision and accuracy data collection is 70-130%.

3.16.1.2 Horwitz intra-laboratory values are used as a guideline for determining reproducibility acceptability. The laboratory shall indicate any compounds that they feel are not acceptable and/or those that should be classified as Marginal Performing Analytes. These laboratory recommendations are subject to approval by WSDA.

3.16.1.3 Analytes that do not meet method validation criteria for linearity, calibration integrity, ion ratios, recovery (individual or mean), or precision (%CV) shall be reported to WSDA with the method validation. Rather than not including them in the laboratory's screening list, WSDA and the laboratory may decide that marginal data are preferable to no data. These compounds shall be designated as Marginal Performing Analytes.

3.16.2 Laboratory Control Sample Criteria

3.16.2.1 All spiked compounds shall have recoveries between 70 and 130%, within the statistically calculated range, or within a range agreed upon with WSDA.

3.16.2.2 If a large number of analytes are in the spike, it becomes statistically likely that a few will be outside control limits. This may not indicate that the system is out of control. The laboratory shall have written criteria for when corrective action(s) will be necessary.

3.16.2.2.1 Some analytes may not be optimally recovered during method validation trials. Recoveries may be low and/or erratic and rather than not including them in the laboratory's screening list, the laboratory may consult with WSDA to determine if marginal data may be preferable to no data. If reported by the laboratory, the codes for Marginal Performing Analytes shall be utilized. Once a compound is designated as a Marginal Performing Analyte, that designation shall not be changed unless approved by the WSDA.

3.16.2.2.2 Some analytes that behave acceptably during method validation may behave unacceptably during the analysis of routine batches. This may be due to the fact there is more commodity variability among actual samples than there is in the limited matrix utilized for method validation batches. As above, rather than dropping these analytes from the screening list, the laboratory should consult with WSDA to determine if they should be reclassified as Marginal Performing Analytes. If a compound is reclassified as a Marginal Performing Analyte, an e-mail notification to the WSDA shall be sent and approved/acknowledged, and that designation shall not be changed unless an e-mail communication is sent by the WSDA reversing the previous approval.

3.16.3 Response to Failure to Meet Laboratory Control Sample Criteria

If a spike analyte fails, even after re-injection/re-aliquoting, it cannot be reported. When a spiked pesticide recovery falls outside the range criteria, any one of the following options, or combination thereof, may be chosen by the Scientific Director or designee.

3.16.3.1 The original extract may be re-injected or re-aliquoted. If the spiked pesticide recovery falls within the range criteria, then the results from the re-injected extract shall be reported.

3.16.3.2 The sample set may be re-extracted from the frozen homogenate. If the spiked pesticide recovery falls within the range criteria, the rerun results shall be reported.

3.16.3.3 Other options may be acceptable depending on the outcome of investigations and/or consultations with WSDA.

3.16.4 Process Control Criteria

Each laboratory shall decide whether to use the Absolute Range Criteria or the Statistically Calculated Range Criteria. A laboratory may choose different Range Criteria for different test types, but it is intended that a laboratory stay with the chosen criteria unless approved by the laboratory QA Officer.

3.16.4.1 Absolute Range

Each process control recovery shall fall between 70-130% for all detection systems used to calculate sample data.

3.16.4.2 Statistically Calculated Range

The mean recovery for a sample set's process control shall be calculated. Each process control recovery shall fall within its acceptance recovery range, which is the mean recovery plus and minus three standard deviations.

3.16.5 Response To Failure To Meet Chosen Process Control Criteria Range

If a process control fails, even after re-injection/re-aliquoting/re-extraction, the results may be reported, based on best professional judgment.

When a process control falls outside the chosen range criteria, any one of the following options, or combination thereof, may be chosen by the scientific director or designee.

3.16.5.1 The original extract may be re-injected or re-aliquoted. If the process control recovery falls within the chosen range criteria, then the results from the re-injected or re-aliquoted extract shall be reported.

3.16.5.2 The sample may be re-extracted from the frozen homogenate. If the process control recovery falls within the chosen range criteria, the re-run results shall be reported.

3.16.5.3 The original results may be reported with an explanation (e.g., pipette error, the PC recovery exceeds 130% but all analytes in the sample are non-detects, etc.). The laboratory shall ensure that reported data is not compromised and the explanation shall be conveyed to the WSDA (e.g., note in reporting laboratory information system, written explanation in data pack).

3.16.5.4 Other options may be acceptable depending on the outcome of investigations and/or consultations with WSDA. An explanation shall be conveyed to the WSDA.

3.16.6 Evaluation of Recoveries

Laboratories shall use control charting or other appropriate statistical tools to evaluate recoveries on a set-to-set basis and monitor trends over time.

3.17 Proficiency Testing

Proficiency testing requirements set by WSDA shall be followed.

3.18 Measurement Uncertainty

Certified labs are responsible for evaluating measurement uncertainty using appropriate practices and protocols. Appropriate guides and resources for evaluating measurement uncertainty include: The Joint Committee Guides in Metrology "Evaluation of measurement data – Guide to the expression of uncertainty in measurement (GUM)", ISO/IEC Guide 98, or EURACHEM/CITAC Guide "Quantifying Uncertainty in Analytical Measurements. Additional methods for calculating measurement uncertainty may be utilized with the approval of the WSDA.

Certified labs shall submit reports to the client annually or report measurement of uncertainty on their website available to the WSDA and clients.

4 References

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5 Acknowledgments

The above method was adapted from the United States Department of Agriculture Pesticide Data Program by the Cannabis Laboratory Analysis Standards Program to meet the recommendations of the Cannabis Science Task Force as procedures for determining pesticide residues for accredited cannabis laboratories in Washington state.