Cannabis Pesticide Data Analysis

1.0 Scope and Application

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

1.2 To provide pesticide procedures for:
- 1.2.1 Instruments, equipment, and injection sequence used in Washington state accredited cannabis testing laboratories.
- 1.2.2 Quantitative and qualitative analysis of pesticide residues.
- 1.2.3 Data reduction, reporting, and submission by participating laboratories.

1.3 These standards must be followed by all laboratories conducting pesticide residue studies for cannabis samples, including support laboratories.

2.0 Outline of Procedures

3.0 Instrumentation

3.1 SOPs and Manuals

Each laboratory shall develop SOPs for equipment operation. The SOPs shall set forth in sufficient detail the methods, materials, and schedules to be used in the routine inspection,

cleaning, maintenance, testing, calibration, and/or performance verification of equipment used, and shall, when appropriate, specify remedial action to be taken in the event of failure or malfunction of equipment. SOPs and operator manuals shall be readily accessible to applicable laboratory staff. Manufacturer's manuals or published literature may be used only as supplements to SOPs.

3.2 Maintenance

All instruments and other equipment used in the analysis of cannabis samples shall be inspected, cleaned, and maintained in proper working condition to ensure the accuracy, precision, and sensitivity requirements specified in this standard and rules are met.

3.3 Performance Verification

Before being placed into service, an instrument shall undergo appropriate checks to establish that all requirements are met. See CLASP Method 982 – Pesticide QC Processing.

3.4 Records

3.4.1 Records (e.g., logbooks) shall be maintained for all critical equipment and instruments. These records shall be used to document all routine and non-routine inspection, maintenance, and calibration activities, including the date, the identity of the personnel performing the activities, and any maintenance (routine or otherwise), repairs, or remedial actions.

3.4.2 Data packages shall reflect the specific instruments and equipment that were used to generate, measure, or assess the data. Data on the performance verification of instruments (e.g., gas chromatograph-mass selective detector (GC-MSD), etc.) utilized in the analysis of a data set are to be maintained by the laboratory. See Section 8 of this document for hardcopy data package requirements. See Section 7 of this document for mass spectrometry (MS) documentation requirements.

3.4.3 Calibration and/or performance verification data for balances, refrigerators, and other peripheral equipment do not need to be included in the submitted data packages but shall be maintained by the laboratory.

3.4.4 Records shall be stored for at least five years.

4.0 Calibration

4.1 Calibration Integrity

Instruments and equipment that have significant effects on test results shall be calibrated at the minimum frequency specified in the laboratory's internal SOPs.

4.1.1 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 to evaluate the variability in instrument response and any changes in retention time (see 4.1.2). Injection of a standard(s) between the beginning and end of a run may also be required. Calibration integrity shall be calculated in terms of relative percent difference (RPD), percent difference (%D), or percent relative

standard deviation (%RSD) using the following equations:

$$RPD = \frac{|X_1 - X_2|}{\left|\frac{X_1 + X_2}{2}\right|} * 100$$

Where X1 is the response of the first analytical standard injected and X2 is the response of the second standard injected;

$$\%D = \frac{C_1 - C_2}{C_1} * 100$$

Where C1 is the known concentration of the standard used for quantification and C2 is the concentration of that standard calculated using the calibration curve;

$$\%RSD = \frac{SD}{Avg.RF} * 100$$

Where SD is standard deviation;

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

And RF is response factor, or the area or height of each standard divided by the concentration of that standard. Most instrument software can be set to flag this criteria when out of range.

4.1.2 Standard response drift greater than 20% RPD, %D, or RSD indicate that additional standards within the run may be injected in order to attempt to meet the 20% calibration integrity requirement. Each laboratory shall document exceptions in internal SOPs and shall determine the number of intermediate standards required throughout the run to maintain calibration integrity.

4.1.3 For cases where no residues were detected in samples and only the spike recovery is being quantified, the requirement for calibration integrity shall be 30%.

4.2 Quantification Using Calibration Curves

4.2.1 Incurred residue(s) may be subtracted from matched standards prior to generating the calibration curve. A laboratory may elect to subtract incurred residue(s) if the following conditions are met:

- Blank matrix cannot be obtained. The laboratory shall make every effort to obtain blank matrix such as purchasing cannabis from a retailer, saving analyzed samples that are pesticide free, etc.
- The incurred residue is less than 2xLOQ (Limit of Quantitation).

If a laboratory elects to subtract incurred residues from matrix matched standards, they shall have internal procedures on how to handle the subtraction process.

4.2.2 Calibration curves shall be constructed using standards which bracket the expected range of residue concentration. A calibration curve must include no less than four points, use a linear fit, use no weighting (1/X or 1/C are acceptable), and do not force the curve through zero.

4.2.3 For any analyte that is quantitated using a calibration curve, the fitness of curve, shall be demonstrated in the same injection sequence used to report the data by one of the following accepted methods:

- correlation coefficient (where R > 0.9975 / R2 > 0.995),
- percent relative standard deviation (where %RSD ≤ 20), or
- percent difference of calculated vs. known standard concentration in the curve (where %D is within 20%).

4.2.4 The laboratory shall specify in an internal SOP the method/parameter(s) used to demonstrate fitness of curve.

4.2.5 Results obtained using a calibration curve shall lay within the range of the calibration curve. If results fall outside the calibration curve, the sample must be diluted or the calibration curve extended. The procedure for extending the range of the 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 QA Manager (Quality Assurance Manager).

If method range has been extended beyond the highest validated level, then samples may be diluted for quantitation purposes. However, dilutions must be done proportionally with matrix so that the matrix concentration of the sample is similar to that of the analytical standards used to prepare the calibration curve.

4.3 Quantification of Multi-Peak Compounds

Quantification of multi-peak compounds may be based on the largest peak or the sum of all the peaks. Summation using the instrument's peak integration software is preferred and, when used, must be applied to the multi-peak compound with consistent parameters across all samples. Otherwise, when reporting multi-peak compounds as total (combined) values and one or more peaks, but not all, are Below Quantifiable Level (BQL), determine and report the value(s) for the BQL peak(s) using the value calculated by the data station based on the calibration table. If one or more peaks are less than the Limit of Detection (LOD), or LOQ where LOD=LOQ, do not include them in calculating the total (combined) value.

4.4 Quantification of Spikes

4.4.1 Incurred residue(s) may be subtracted from spike recovery(ies) prior to calculating the percent recovery. A laboratory may elect to subtract incurred residue(s) if the following conditions are met:

- Blank matrix cannot be obtained. The laboratory shall make every effort to obtain blank matrix such as purchasing organic materials, saving analyzed samples that are pesticide free, etc.

- The incurred residue is less than 2xLOQ.
- The laboratory shall record blank subtracted spike recovery data.

If a laboratory elects to subtract incurred residues, they shall have internal procedures on how to handle the subtraction process.

4.4.2 Incurred residues, as determined using the matrix blank, shall not be subtracted from the spike when the residue in the matrix blank exceeds 2xLOQ. If an incurred residue is greater than 2xLOQ.

4.4.3 Pesticides not recovered in the quality control spikes shall not be reported.

5.0 Generating Raw Data

5.1 Injection Sequence Description

5.1.1 Each laboratory shall develop an SOP detailing an appropriate injection sequence to ensure data integrity and uniform response across the sample set. “Uniform response” shall be construed as no greater than 20% RPD, %D, or RSD between calibration responses (refer to Section 4.1 of this document) or 30% if a residue was not detected and only the spike is being quantitated.

5.1.2 Standards for each compound analyzed shall be included with every injection sequence. It is recommended that standards spanning the expected range of residue concentrations, such as 1xLOQ to 10xLOQ, be included in the sequence to allow construction of a calibration curve.

5.1.3 Standards must be run at a minimum of the beginning and end of the data run to demonstrate calibration integrity. This may be accomplished via a single standard or a full set of calibration curve standards.

5.1.4 Each initial analytical run shall include the reagent blank, matrix blank, spikes, and samples. For additional runs (i.e., reinjects/dilutions) QC samples shall be run as necessary (i.e. reagent or matrix interference).

5.2 Retention Time Criteria (Selective Detection and MS Systems)

5.2.1 GC and LC Retention Time

5.2.1.1 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 0.1 minutes.

5.2.1.2 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 minutes.

5.2.2 MS Screening for Identification

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 quantification 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.

5.3 MS Confirmation Criteria

5.3.1 GC/MS and LC/MS Confirmation Criteria

5.3.1.1 A minimum of three structurally significant ions (meeting the 3:1 s/n ratio) are required for confirmation. For GC/MS, because the molecular ion is the most structurally significant ion in a mass spectrum, if it is present and meets the 3:1 s/n ratio, it is preferable that it be included as one of the three ions.

Note: If instrument conditions and/or ionization techniques limit the number of ions available, the laboratory shall request a deviation from WSDA in order to report results under these conditions.

5.3.1.2 A pair of isotopic cluster ions may be used as two of the three structurally significant ions required for confirmation.

5.3.1.3 Use of fragment ions resulting from water loss to meet the three structurally significant ions requirement is discouraged.

5.3.1.4 The confidence limits of the relative abundance of structurally significant ions used for SIM and/or full scan identification shall be ± 30% (relative) when compared to the same relative abundances observed from a standard solution injection made during the same analytical run.

5.3.1.5 MS spectra produced by “soft” ionization techniques (e.g., GC/MS - chemical ionization and for LC/MS – APCI, APPI, ESI, etc.) may require additional evidence for confirmation. If the isotope ratio of the ion(s) or the chromatographic profile of isomers of the analyte is highly characteristic, there may be sufficient information for confirmation. Additional evidence may consist of MS/MS data, use of a different ionization technique, use of a different chromatographic separation system, and for LC/MS systems, altering fragmentation by changing ionization conditions.

5.3.1.6 GC/MS: Fragmentation that results from “soft” ionization techniques is highly dependent on instrument design and the conditions applied (i.e., the obtained spectra can widely differ). Commercially available spectral libraries bundled with GC/MS instruments may contain spectra generated under standard 70eV EI conditions; therefore, the use of library search software for spectra from “soft” ionization techniques could result in identification errors and is discouraged.

5.3.2 GC/MS/MS and LC/MS/MS Confirmation Criteria

5.3.2.1 Target analyte confirmation shall be performed by either (1) monitoring the

transition of one precursor ion to at least two product ions, OR (2) monitoring at least two precursor-to-product ion transitions.

Multipeak compound confirmation may be based on the largest peak or the sum of all the peaks. If it is based on the sum of all the peaks, one or two of the constituents can be used for both transitions.

Note: If instrument conditions and/or ionization techniques limit the number of transitions available, the laboratory shall request a deviation from WSDA in order to report results under these conditions.

5.3.2.2 The abundance of the signal from the precursor-to-product ion transition shall meet the 3:1 s/n ratio requirement.

5.3.2.3 The relative abundances of ion transitions used for compound identification in the sample shall be ± 30% (relative) when compared to the same relative abundances observed from a standard solution analyzed during the same analytical run if more than one precursor-to-product ion transition is monitored. The ion ratio tolerance shall be calculated using the following example: If the ion ratio (qualifier area count/target area count) is 15%, the acceptable range will be 15%+/-4.5 or 10.5% to19.5%.

5.3.2.4 Use of product ions resulting from water loss for identification is discouraged.

Note: Any information that provides a contraindication of identity of the residue will be addressed in the internal SOP by the laboratory.

5.4 **MS Documentation Criteria**
Structurally significant ions and/or precursor-to-product ion transitions used for confirmation shall be documented.

6.0 Data Handling

6.1 Raw Data Handling

6.1.1 Hardcopy raw data are defined as any laboratory worksheets, logbooks, records, notes, chromatograms, calculations, instrument printouts, and any other data, which are the result of original observations and activities. Electronic raw data are the files generated by the instrument system.

6.1.2 For manual entry, hardcopy raw data shall be recorded directly, promptly, and legibly in permanent ink. Pencil or erasable pen is not acceptable. All data entries shall be dated on the date of entry and signed or initialed by the person entering the data. Each individual error shall be corrected using a single-line cross out (no white-out). It is recommended, but not required, that the reason for the correction be indicated. Each correction shall be dated and initialed. Documented error codes may be used to explain errors. Correction of multiple errors may be accomplished in the following manner:

6.1.2.1 On first occurrence of the error, or on a summary sheet, make/indicate the appropriate correction, including date, initials, explanation of error/error code, and all affected subsequent entries.

6.1.2.2 Each subsequent occurrence of the error must then be corrected, dated, and initialed.

6.1.3 Each participating laboratory shall ensure sample and data traceability for raw and electronic data collection and processing. Chromatograms that have been reprocessed through the data system shall be clearly labeled.

6.1.4 Each participating laboratory shall maintain a log of names, initials, and signatures for all individuals who are responsible for signing or initialing any laboratory record.

6.2 Data Package Requirements

6.2.1 Routine sample data packages, PT data, and method validation data packages retained by the certified laboratory shall consist of laboratory records (i.e., worksheets and/or completed forms), sample manifests (where applicable), and supporting technical data in the form of chromatograms and integration reports, calculations, and derived data. Data requirements consist of two types, instrument and chromatographic. The following information shall be included in the data package.

6.2.1.1 The instrument method shall be included or referenced. Instrument information shall be traceable. Examples may consist of instrument type and identifier, detector type, injection volume, temperature parameters (injector, detector, oven), analytical column parameters (phase, film thickness, diameter, length), and instrument parameters (integration threshold, attenuation, timed events).

6.2.1.2 Chromatographic information shall be traceable. Examples may consist of sample ID, analyst name, dilution information, and date and time of injection.

6.2.2 At a minimum, hardcopies or locked, traceable, and verifiable electronic copies of data sets shall include the following:

6.2.2.1 Instrument methods or references to them (data acquisition, calibration/standardization, and data analysis parameters)
6.2.2.2 Injection sequences
6.2.2.3 Chromatograms and/or instrument reports of samples, standards, reagent blanks, matrix blanks, and laboratory control sample
6.2.2.4 Any documents submitted with the sample
6.2.2.5 Matrix blank, reagent blank, laboratory control sample, and sample results
6.2.2.6 Documentation of technical and QA review

Note: Laboratories that choose to retain electronic data sets as PDF or Excel files shall ensure all requirements for QA, traceability, etc. are met. Nothing shall be lost in the electronic domain that would normally be captured on paper, and all markups

of the original chromatogram shall also be retained.

6.2.3 Method validation data packages shall only be submitted with prior approval and shall include copies of the summary reporting forms, narrative describing the method, and cover memo submitted to WSDA.

7 Data Reporting

7.1 Calculations and Significant Figures

7.1.1 Each laboratory shall have an internal SOP describing the data processing steps taken to reach the final reported concentration. Data shall not be ignored without a written explanation (e.g., instrument malfunction, wrong standard used, co-eluting peak, etc.).

7.1.2 In calculations, at least one significant figure in excess of the reporting requirements shall be carried through the calculation. When rounding is required, values greater than or equal to 5 shall be rounded up.

7.1.3 Percent recoveries shall be reported to two significant figures if less than 100 or to three significant figures if greater than 100.

7.1.4 Concentrations shall be reported to two significant figures in parts per million (ppm).

7.1.5 Individual peaks may be reported for multiple peak compounds. If separate standards are available for separate isomers, it is preferable to report the isomers separately.

7.2 Determination of Residue Concentrations for Reporting Purposes

7.2.1 A laboratory may elect to set LOD = LOQ provided all of the following conditions are met:

- the analyses are completely performed via MS systems (i.e., quantification and self- confirmation) and
- the qualifier ions are at least 3 x s/n and
- the quantification ions have a response at least 10 x s/n.

7.2.2 Do not report residue concentrations less than the verified LOQ.

7.3 Action Limits Table

LCB maintains a list of action levels for recreational cannabis.

Analyte	µg/g (ppm)	CAS#
Abamectin (Sum of Isomers)	0.50	71751-41-2
• Avermectin B1a		65195-55-3
• Avermectin B1b		65195-56-4
Acephate	0.40	30560-19-1
Acequinocyl	2.0	57960-19-7
Acetamiprid	0.20	135410-20-7

Aldicarb	0.40	116-06-3
Azoxystrobin	0.20	131860-33-8
Bifenazate	0.20	149877-41-8
Bifenthrin	0.20	82657-04-3
Boscalid	0.40	188425-85-6
Carbaryl	0.20	63-25-2
Carbofuran	0.20	1563-66-2
Chlorantraniliprole	0.20	500008-45-7
Chlorfenapyr	1.0	122453-73-0
Chlorpyrifos	0.20	2921-88-2
Clofentezine	0.20	74115-24-5
Cyfluthrin	1.0	68359-37-5
Cypermethrin	1.0	52315-07-8
Daminozide	1.0	1596-84-5
DDVP (Dichlorvos)	0.10	62-73-7
Diazinon	0.20	333-41-5
Dimethoate	0.20	60-51-5
Ethoprophos	0.20	13194-48-4
Etofenprox	0.40	80844-07-1
Etoxazole	0.20	153233-91-1
Fenoxycarb	0.20	72490-01-8
Fenpyroximate	0.40	134098-61-6
Fipronil	0.40	120068-37-3
Flonicamid	1.0	158062-67-0
Fludioxonil	0.40	131341-86-1
Hexythiazox	1.0	78587-05-0
Imazalil	0.20	35554-44-0
Imidacloprid	0.40	138261-41-3
Kresoxim-methyl	0.40	143390-89-0
Malathion	0.20	121-75-5
Metalaxyl	0.20	57837-19-1
Methiocarb	0.20	2032-65-7
Methomyl	0.40	16752-77-5
Methyl parathion	0.20	298-00-0
MGK-264	0.20	113-48-4
Myclobutanil	0.20	88671-89-0
Naled	0.50	300-76-5
Oxamyl	1.0	23135-22-0
Paclobutrazol	0.40	76738-62-0
Permethrins (Sum of Isomers)	0.20	52645-53-1
• cis-Permethrin		54774-45-7
• trans-Permethrin		51877-74-8
Phosmet	0.20	732-11-6
Piperonyl butoxide	2.0	51-03-6
Prallethrin	0.20	23031-36-9
Propiconazole	0.40	60207-90-1
Propoxur	0.20	114-26-1
Pyrethrins (Sum of Isomers)	1.0	8003-34-7

• Pyrethrin I		121-21-1
• Pyrethrin II		121-29-9
Pyridaben	0.20	96489-71-3
Spinosad (Sum of Isomers)	0.20	168316-95-8
• Spinosyn A		131929-60-7
• Spinosyn D		131929-63-0
Spiromesifen	0.20	283594-90-1
Spirotetramat	0.20	203313-25-1
Spiroxamine	0.40	118134-30-8
Tebuconazole	0.40	80443-41-0
Thiacloprid	0.20	111988-49-9
Thiamethoxam	0.20	153719-23-4
Trifloxystrobin	0.20	141517-21-7

8.0 Data Review

8.1 Each data package shall undergo review by the technical and certifying scientist for accuracy and completeness, adherence to WSDA criteria, and integrity of the overall quality system. The certifying scientist shall have access to all documentation necessary to achieve this objective. Both technical and certifying scientist reviews shall be documented.

8.2 Following both reviews of a data package, that data shall not be changed by any laboratory personnel unless as a response to comments/concerns/recommendations by the certifying scientist. Actions taken as a result of technical and/or certifying findings shall be documented.

9.0 References

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10.0 Acknowledgements

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.