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GUIDE TO HARMONIZING CANNABIS LABORATORY QUALITY AND TESTING PRACTICES

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INTRODUCTION

Laboratories testing cannabis, cannabis-based products and hemp play an important role in ensuring public safety, improving product quality and consistency, as well as consumer satisfaction. Providing accurate and traceable quantitative and qualitative data, laboratories ensure that cannabis and hemp regulators and consumers are provided information to properly regulate and purchase cannabis or cannabis-based products regardless of the state in which they are purchased.

The Guide to Harmonizing Cannabis Laboratory Quality and Testing Practices was developed to recommend guidance for a consistent and science-based approach to regulatory processes by describing basic quality assurance requirements for the laboratories testing marijuana and/or hemp plant materials and their derivatives across the nation. The document was developed with the aid of industry experts and stakeholders including laboratories, accreditation bodies, and input from state regulatory bodies. The document is meant to be a living resource to support the growing cannabis and hemp industries and encourage a level playing field with regard to quality management systems, technical quality control, and method requirements for testing laboratories.

The recommendations and guidance in this document do not supersede accreditation requirements at the federal, state, or local levels. The guidance document is a collection of science-based best practices, which are common to other testing industries, as they apply to cannabis and hemp testing laboratories. The document was written to be harmonized with IS17025:2017 and includes interpretation of the standard with respect to cannabis testing. The compliance guidance is also derived from good laboratory practices (GLP) and good manufacturing practices (GMP). Though this document is guidance the following terms are used:

- “must” to represent a requirement which shall be applied as written
- “should” to represent a recommendation which can be modified when necessary

Each laboratory must evaluate, develop, and implement the appropriate safety, health and environmental standard operating procedures based on the local, state and federal regulatory requirements specific to the scope of work being performed within the laboratory. These items are outside the scope of this document. The security guidance provided in this document may not be sufficient to meet the regulatory guidance requirements under which the laboratory may be operating, however it is the responsibility of the individual laboratory to ensure it is meeting the federal, state, local security regulation requirements.

SCOPE

The Guide to Harmonizing Cannabis Laboratory and Quality Testing Practices provides recommendations for laboratories and regulators nationwide as the essential minimum elements of laboratory quality to make certain accurate, consistent, traceable, and defensible data are delivered which meet public safety and regulatory requirements. It should be noted that these are meant to be the minimum requirements. Users may choose to exceed these requirements at their discretion.

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The guide may be applied to all sizes of laboratories to ensure quality basics are met for the analyses performed by the laboratory. The guide is a reference which can be utilized by accrediting bodies, customers, or regulatory bodies to confirm quality assurance objectives are being met and are harmonized among all states in which cannabis and hemp testing programs have been implemented in support of their regulatory programs.

DEFINITIONS/TERMINOLOGY

Cannabis: A genus of flowering plants in the family Cannabaceae. This includes both "Hemp" and "Marijuana" as defined below. ILI definition

Cannabis-derived product: Product other than cannabis itself that contains or is derived from cannabis. ILI definition

Cannabis-derived compounds: Cannabis and cannabis-derived compounds that may be used in drug manufacturing include botanical raw materials, extracts, and highly purified substances of botanical origin. This guidance does not address development of fully synthetic versions of substances that occur in cannabis, sometimes known as cannabis-related compounds, which are regulated like other fully synthetic drugs.(1)

Cannabinoids: Distinctive class of compounds that are capable of interacting with the specific receptors of the endocannabinoid system in the human body. ILI definition

Corrective and Preventive Action (CAPA): A laboratory system implemented to collect information, analyze information, identify and investigate quality problems, and take appropriate and effective corrective and/or preventive actions to prevent their recurrence.(2)

Certified Reference Material (CRM): Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a reference material certificate issued by an authoritative body that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.(3, 4)

Critical Supplies: A consumable, reference material, or service utilized by a laboratory that has a direct impact on the final reported result.(5)

Decision Rule: A rule that describes how measurement uncertainty is accounted for when stating conformity with a specified requirement. (6)

Demonstration of Capability: A procedure to establish the ability of the analyst to perform analyses with acceptable accuracy and precision. (7)

Hemp: (a) For the purposes of 7 CFR part 990, and as defined in the 2018 Farm Bill, the term "hemp" means the plant species Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis.(8)

The plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis." 7 U.S.C. 1639o(1). Pursuant to the amended definition, cannabis plant material which contains 0.3 percent or less delta-9 tetrahydrocannabinol (THC) on a dry weight basis is not a controlled substance and does not require a DEA registration to grow.(9)

Laboratory control sample (LCS) (however named, such as laboratory fortified blank, spiked blank, or QC check sample): A portion of appropriate clean matrix that is spiked with known quantities of target analytes and carried through the entire sample preparation process, and treated exactly as a sample, including exposure tall glassware, equipment, solvents, and reagents that are used with other samples. The LCS measures the accuracy of the methodology. The LCS may be prepared from the same source as the calibration standards, or from a second source. (10)

Laboratory Operation: Person, group of persons, or business entity that conducts analytical testing of cannabis and cannabis-derived products (This may include performance of work outside the permanent facility). (11)

Limited Access Area: An area in which cannabis or cannabis products are stored or held and is only accessible to a licensee and authorized persons.(12)

Limit of Detection (LOD): Is defined as the lowest concentration or mass of analyte in a test sample that can be distinguished from a true blank sample at a specified probability level.(13)

Limit of Quantitation (LOQ): Minimum concentration or mass of analyte in a given matrix that can be reported as a quantitative result.(14)

LIMS: Laboratory Information Management System that may be electronic, hardcopy, or some combination of electronic and hardcopy.(15)

Marijuana: 1. all parts of the plant Cannabis sativa L., whether growing or not; the seeds thereof; the resin extracted from any part of such plant; and every compound, manufacture, salt, derivative, mixture, or preparation of such plant, its seeds or resin. Such term does not include the mature stalks of such plant, fiber produced from such stalks, oil or cake made from the seeds of such plant, any other compound, manufacture, salt, derivative, mixture, or preparation of such mature stalks (except the resin extracted therefrom), fiber, oil, or cake, or the sterilized seed of such plant which is incapable of germination." 21 U.S.C. § 802(16).(16)

Matrix: The components of a sample other than the analyte. (17)

Matrix-Specific Quality Control Sample: Real, thoroughly characterized in-house sample that is run once a day to track accuracy and precision; should record values in a control chart for monitoring. The sample can also be used for inter-instrument comparisons. Ideal variance (measured by %RSD) should be less than 5%. ACIL definition

Method Blank: A quality system matrix that is similar to the associated samples and is known to be free of the analytes of interest. (18)

Security Monitoring: Continuous and uninterrupted attention to potential alarm signals that could be transmitted from a security alarm system located at the laboratory premises for the purpose of summoning a law enforcement officer to the premises during alarm conditions. (19)

Nonconforming Work (Nonconformances): when any aspect of its laboratory activities or results of this work do not conform tits own procedures or the agreed requirements of the customer (e.g. equipment or environmental conditions are out of specified limits, results of monitoring fail to meet specified criteria) (20)

Reagent Blank: A sample without matrix, prepared identically to a field sample (i.e. same glassware, solvents, reagents, etc.). The purpose of a reagent blank is to identify any possible sources of contamination in the reagents, equipment, glassware or laboratory environment. ACIL definition

Phytocannabinoid: Cannabinoid chemical compounds found in the cannabis plant. (21)

Sequestered Microbial Sample: A representative subsample of the total sample batch which is incrementally sampled from within the sample batch and sequestered prior to homogenization then homogenized separately in a manner that does not affect the microbial load present in the sample prior to testing. ACIL definition

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Standard Reference Material (SRM): A CRM issued by NIST that also meets additional NIST-specific certification criteria and is issued with a certificate or certificate of analysis that reports the results of its characterizations and provides information regarding the appropriate use(s) of the material (NIST SP 260-136). Note: An SRM is prepared and used for three main purposes: (1) to help develop accurate methods of analysis; (2) to calibrate measurement systems used to facilitate exchange of goods, institute quality control, determine performance characteristics, or measure a property at the state-of-the-art limit; and (3) to ensure the long-term adequacy and integrity of measurement quality assurance programs. The term "Standard Reference Material" is registered with the United States Patent and Trademark Office. (4)

Tamper-Evident Device: A device or procedure which makes unauthorized access to protected objects easily detectable (22)

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21. Production and/or Use of Chemically Modified or Converted Industrial Hemp Cannabinoids. CDPHE Notice Letter. May 14, 2021.

22. Rule Number 64ER20-1, Certified Marijuana Testing Laboratory Rules, Definitions (68), State of Florida, January 22, 2020

Note: ACIL and ILI have defined some terms used in this document where another suitable definition could not be found in the public domain.

LABORATORY QUALITY MANAGEMENT SYSTEM

1. Organization and Scope of Work

The laboratory must be registered as a legal business operating within and compliant with federal, state, and local regulations.

The laboratory must define and document activities that occur within the laboratory and activities that are externally outsourced as part of adhering to this document and federal, state, and local regulations.

The laboratory must define its management structure and the responsibilities of its personnel in terms of customer support, analytical, and administrative operations.[a]

The laboratory management must have the responsibility and authority to establish, implement, and control documented procedures for laboratory activities to meet the needs and requirements of customers, regulators, and accreditation bodies.[a]

The laboratory must develop, control, and communicate the importance of standard operating procedures (SOPs) that must be followed for laboratory activities and improvement of processes.[a]

The laboratory must have a quality manual that documents and references quality-related procedures and SOPs. The contents of the quality manual must include the following to ensure that the quality, applicability, quality to meet regulatory and customer quality objectives:

- Policy and procedure references for the quality management system and laboratory operations
- Laboratory activities, goals, objectives, employee responsibility and accountability
- Ensure that employees know the importance of following SOPs, policies, and other related procedures.
- Ensure impartiality and confidentiality is incorporated into all employee training.

[a] Based on IS17025:2017(E) Section 5 Structural Requirements

2. Laboratory Personnel Requirements

The laboratory must define roles for personnel who have the responsibility and authority to carry out the following essential functions within the management system: [b]

- Manage laboratory activities to ensure compliance, reduce and manage occurrences of non-conformances, seek continual process improvement and effectiveness to the quality management system. [b]
- Ensure that implementation of the quality management system is effective and adheres to the requirements of its customers and federal, state, and local regulations. [b]

The laboratory must document and maintain the professional requirements for each position within the laboratory in terms of education, training, authorization to perform work, and effectiveness of training through documented demonstration of capability.

The laboratory must determine and implement a program to ensure the continued effectiveness of training through a process of continuing demonstration of capability.

The laboratory must document training specifically in the areas of safety, hazard, and emergency response. The laboratory must supply the appropriate information tallow employees to follow local, state, and federal regulatory requirements and understand their importance to the position of the employee.

[b] Based on IS17025:2017(E) Section 6.2 Personnel

3. Facility and Security

Facility and Laboratory Environment

The laboratory facility must have appropriate facilities, equipment, and environment to support and perform laboratory activities.

The environmental requirements for laboratory activities must be documented tallow for the laboratory to control, monitor, and record the environmental conditions, as applicable teach scope of testing. The goal of the requirements is to reduce or eliminate contamination, interferences, and/or adverse influences impacting laboratory activities. [c]

[c] Based on IS17025:2017(E) Section 6.3 Facilities and Environment

Laboratory Facilities Security

The laboratory must have the following security measures:

- Video surveillance
- Locks
- Biometric or key card access control for limited-access areas

The laboratory should have a security alarm system installed on perimeter entry points and perimeter windows to ensure premises are continuously monitored and secured.

The laboratory should have video surveillance coverage available at locations of key activities which may include but is not limited to:

- Sample receiving
- Sample storage
- Sample weighing
- Sample destruction

Video surveillance equipment must consist of, at a minimum, digital or network video recorders, video monitors, digital archiving devices, and a printer capable of delivering still photos.

The equipment should have a back-up battery, provide failure notification to designated laboratory staff, and be able to record in all lighting conditions.

Placement of camera(s) should allow for clear identification of any individuals and activities being performed.

Location and Maintenance of Surveillance Equipment

Surveillance recording equipment must be housed in a secured enclosure with access limited to authorized employees, agents of the regulatory authority and state or local law enforcement agencies.

Laboratory management should keep a current list of all authorized employees and service personnel who have access to the surveillance system.

A surveillance equipment maintenance activity log should be maintained and include all service activity including the identity of the individual(s) performing the service, the service date and time, and the reason for service.

Off-site monitoring and video recording storage should meet the requirements of this section.

All surveillance recordings should be kept for a minimum of 45 days.

Surveillance video recordings must not be destroyed if the laboratory management is aware of a pending criminal, civil, or administrative investigation or any other proceeding for which the recording may contain relevant information.

Recordings should be kept in a digital format easily accessed for viewing.

Recordings should be archived in a format that ensures authentication of the recording and guarantees that no alteration of the recorded image has taken place.

The laboratory should ensure that installation, maintenance, and monitoring services meet state requirements.

4. Outside Suppliers of Consumables and Services

External suppliers who are providing consumables or services affecting the quality of the result must be vetted and qualified to be a critical supplier. The laboratory must have procedures and retain records for the qualification of suppliers and, as appropriate, supplier personnel. [d]

The laboratory procedure for vetting vendors and suppliers must include actions to be taken when vendors and suppliers do not meet quality or service requirements (as pre-defined by the laboratory). [d]

The laboratory must have quality specifications for consumables and services that are communicated to the vendors and suppliers. The laboratory must retain records of quality requirements, orders and packing slips to ensure the correct supplies and services are ordered and delivered. [d]

The laboratory must ensure that the quality of critical supplies and services are met prior to using or approving orders for consumables or services. The approval must be performed by personnel who are technically qualified and recorded to ensure traceability to the original material.

Laboratories may subcontract preparation and/or analytical work if allowed under their regulatory requirements. The laboratory must have procedures and records to ensure that the subcontractor is accredited as appropriate to provide the services they are being subcontracted to perform.

All providers must have appropriate certifications and accreditations to the current standards:

- All calibration providers must be ISO/IEC 17025 accredited
- Where commercially available, only IS17034 CRMs or NIST SRMs will be used
- Where commercially available and when applicable to matrix and analyte(s), only 17043 Proficiency Test Providers will be used
- Distribution companies must, at a minimum, be IS9001 certified
- Accrediting bodies must be accredited tIS17011 and signatories to the ILAC Mutual Recognition Agreement (MRA)

[d] Based on IS17025:2017(E) Section 6.6 Externally provided products and services

5. Analytical Service Requests, Invitation for Bid (IFB) and Contracts

The laboratory must have procedures for the review of customer requests and contracts. The procedure must incorporate, at a minimum, the following items:

- The laboratory must understand the purpose of the request as defined and documented by the customer.
- The laboratory must have the capabilities and capacity to accommodate the request. [e]
- The laboratory methodology must be able to meet the data quality objectives, regulatory and quality control requirements needed by the customer.
- The laboratory must provide the customer notification as to when external or subcontract laboratories will be used and have documented approval from the customer. [e]

The laboratory must resolve all differences between the IFB and/or request and the contract with the customer and document the resolution agreed upon. The new contract must be reviewed once the revisions are completed, any changes to the contract must be approved by the customer.

The laboratory must inform the customer of their analytical method capabilities to ensure that the customer data quality objectives are being met. [e]

The laboratory must be cooperative and forthcoming in working with customers to achieve the best outcome for both the laboratory and the customer. The laboratory must have procedures to handle customer requests that may threaten or violate laboratory integrity and impartiality. [f]

All documentation surrounding the customer requests, IFBs, and contracts must be retained and must include but are not limited to: [g]

- Original requests, IFBs, and/or contract [g]
- All communications with the customer [g]
- All changes made to the request or contract [g]
- All approvals from the laboratory and the customer [g]

[e] Based on IS17025:2017(E) Section 7.1.1 Review of requests, tenders and contracts

[f] Based on IS17025:2017(E) Section 7.1.4 Review of requests, tenders and contracts

[g] Based on IS17025:2017(E) Section 7.1.8 Review of requests, tenders and contracts

6. Method Verification and Validation

Method verification and validation are two different activities. Method verification refers to the laboratory establishing their ability to meet the quality control and method specifications of a reference or validated method. A reference or validated method is one that has undergone a validation process by a regulatory body (e.g., state, federal) or a third-party consensus body (e.g., AOAC - *Official Method of Analysis* 2018.11 for cannabinoids in Cannabis plant materials, concentrates, and oils). The process of method verification is to verify that laboratory performance be able to meet the quality control requirements of the method including but is not limited to:

- Limit of detection and quantitation studies
- Initial calibration using a calibration curve and initial calibration verification using a second source material
- Continuing calibration verification, as defined by the method
- Laboratory control spikes or fortified blanks
- Analyst demonstration of competency
- Passing proficiency testing samples in the appropriate matrix, as commercially available.

Method validation refers to the determination that a preparation and analytical process has the ability to meet the sensitivity, selectivity, repeatability, and robustness of data quality objectives with a determined uncertainty. A laboratory developing its own method for use must provide and demonstrate that the process being developed meets predetermined quality control objectives for but not limited to the following aspects below.

- Sensitivity
- Selectivity
- Repeatability
- Reproducibility
- Robustness
- Accuracy
- Linearity
- LOD
- LOQ

Method validation procedures recommended for use are: ASTM D8282-19 Standard Practice for Laboratory Test Method Validation and Method Development, AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, FDA Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products, or IUPAC Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis. These method validation documents have been specifically developed for the cannabis/hemp laboratories or have been in use by the food and botanical industry for many years.

7. Sample Management/Receipt

The laboratory must develop and implement a chain-of-custody process to ensure accurate documentation of the transport, handling, storage, and destruction of samples.

- The chain-of-custody process must require the use of a form containing the following information:
- Laboratory name, physical address, and license number (as required)
- Producer's name, physical address, and license number (as required)
- Where necessary, confirm producer is a legitimate business
- Unique sample identifier,
- Date and time of the sample collection, as available.
- Description and quantity of sample containers
- Amount of sample(s) (e.g. weight, volume)
- Identification of tests requested
- Identification of presence of tamper-evident device, as appropriate
- Printed and signed name(s) of the supplier(s) of sample, unless credentials are captured in the laboratory information management system (LIMS)
- Printed and signed name(s) of the sampler(s), unless credentials are captured in LIMS
- Printed and signed name(s) of the transporter, if different from sampler, unless credentials are captured in LIMS
- Printed and signed name(s) of the testing laboratory employee who received the sample, unless credentials are captured in LIMS
- The chain of custody process may be encompassed within a LIMS or state tracking system such as METRC.
- Description of samples and sample containers received.

Upon receipt in the laboratory, all samples must be compared to the chain-of-custody by a qualified member of the laboratory staff who was not involved with sampling or transportation of the items. All anomalies must be recorded and reported to management and the client upon the recognition of the disparity.

The receiving laboratory must separately document any differences between the quantity specified in the chain-of-custody and the quantities received. Such documentation must be made in any relevant business records and account for the discrepancy.

The laboratory must not accept a sample that is smaller than the standard minimum amount established in regulation or by the laboratory. If a sample is found to be smaller than its standard minimum amount for the analyses requested, the laboratory personnel are required to set it aside, notify the client, and remedy.

Each time the sample changes custody within the laboratory, the date, time, sample weight, and names and signatures of persons involved must be recorded.

Note: This could include but is not limited to when a sample is removed from storage for testing, placed back in storage, or destroyed or disposed.

The laboratory must maintain a record system that facilitates the reconciliation of the sample weight from receipt through destruction or disposal. The laboratory must be able to account for loss.

If any portion of a test or sampling is outsourced, a chain-of-custody meeting the requirements identified above must be implemented.

The chain-of-custody and sample tracking when samples are outsourced to contract laboratories must be done in accordance with state requirements.

8. Technical Records

Technical records must include all records such that a complete audit trail or historical recreation can be developed for the laboratory activities that were critical or determinative to the final reported result(s).

The technical records must include all of the identity(ies) of laboratory personnel who perform activities related to but not limited to, sample receipt, storage, preparation, analysis and disposal of samples. [h]

Observations, data, and calculations originally collected, gathered, or performed must be recorded at the time of their observance or calculation, and must include the identity of the analyst, date, must be legible, and retained per the laboratory's record retention procedure. [h]

If amendments to data, observations or calculations are required, then the following must be recorded, and the original data must be retained:

- The reason for the amendment,
- Identity of the person making the amendment, [h]
- Date of the amendment [h]
- The amendment must be retained per the laboratory's record retention procedure.

[h]Based on IS17025:2017(E) Section 7.5 Technical records

9. Uncertainty

The laboratory must have a procedure and records for the development of measurement uncertainty for the analyses being performed. The laboratory must determine the measurement uncertainty for each analysis performed.[i] The measurement uncertainty must be available for each analytical result should a customer or regulatory body request the information.

If the laboratory performs calibration for in-house equipment, it must provide the uncertainty for these calibrations. [j]

If the measurement uncertainty is explicitly stated in a standard test method in use by the laboratory and the laboratory does not make modifications, verifies they can meet the method's requirements, then the laboratory can assume the method's stated uncertainty.

[i]Based on IS17025:2017(E) Section 7.6.1 Evaluation of measurement uncertainty

[j]Based on IS17025:2017(E) Section 7.6.2 Evaluation of measurement uncertainty

10. Quality Control Data Analysis

The laboratory must have a procedure to track, monitor, and perform statistical data analysis on quality control data generated. The laboratory must perform trend analysis and can use the analysis to improve the quality of the laboratory. The laboratory quality control samples available for use are, but not limited to the below listed.

- Certified reference materials
- Third party quality control reference materials
- Analyte-free matrix blanks
- Laboratory control sample
- Replicate samples
- Proficiency test samples
- Interlaboratory round robin samples

The laboratory must participate in proficiency testing or interlaboratory comparison, as available and appropriate to the matrices the laboratory is analyzing.

11. Reporting Results

The laboratory must have a procedure for the generation and review of final reports prior to being issued to the customer.[k] The final results being reported must be reviewed by the laboratory prior to being released to the customer to ensure accuracy and completeness. The final issued report must be retained in accordance with the record retention procedure established by the laboratory. [l]

The final report must contain, but is not limited to, the following elements:

- Laboratory name, address, and contact information[m]
- Name and title of the individual releasing or issuing the report
- A unique identifier or revision number for the report[m]
- Unique sample name[m]
- Customer contact information [m]
- Methods used for the preparation and analysis of the samples[m]
- Preparation and analysis dates for each sample and analysis
- Report issue date
- Results with units as determined by the laboratory
- Description of the sample received
- Any deviations or changes made to the methods or agreements established for the analysis of the samples
- Any non-conformances accompanied by a statement describing how they affected the quality of the results
- Decision Rules, if statements of conformity are reported

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The laboratory must record and make available the following information if requested:

- Sampling plan if the sampling was performed by the laboratory[m]
- The results of all Quality Assurance and Control samples associated with sample batches
- Measurement Uncertainty [m]
- Photograph of the sample(s)

Any amendments made to the final report must be noted and include, but not limited to the following:

- New revision identifier or report number[n]
- Reason for the amendment
- What portion of report was amended[n]
- Revision date. [n]

Both the amended and original report must be retained by the laboratory in accordance with the laboratory's record retention procedure.

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[k]Based on IS17025:2017(E) Section 7.8.1.1Reporting results

[l]Based on IS17025:2017(E) Section 7.8.1.2 Reporting results

[m]Based on IS17025:2017(E) Section 7.8.2.1 Common requirements for reports (test, calibration, or sampling)

[n]Based on IS17025:2017(E) Section 7.8.8 Amendments to reports

12. Customer Service/Feedback/Complaints

The laboratory should consider the data quality objectives of the customer when proposing which analytical services to offer for a particular scope of work. The laboratory should cooperate and work with customers to ensure customer needs are understood and documented. [o]

The laboratory must seek customer feedback annually related to the quality of their service performance, results package and products delivered to the customer.

The laboratory must have procedures and records to document the following steps concerning complaints: [o]

Acknowledge and document the complaint completely to ensure that the complaint is understood, [o]

The complaint is investigated for validity and accuracy to ensure that the complaint is addressed and resolved, [o]

Investigations must be overseen by management [o]

Investigations must be conducted by personnel other than those involved in initial test/processing

Review should, whenever possible, include empirical evidence/data to support the laboratory's conclusion(s)

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[o]Based on IS17025:2017(E) Section 7.9 Complaints

13. Nonconforming work

Nonconforming work is the occurrence of laboratory activities that do not conform to the quality management system, customer requirements, and/or regulatory requirements. The laboratory must have procedures and retain records for the investigation of nonconforming work. The laboratory must investigate the characteristics and inherent issues of the nonconforming work and determine scope of the nonconforming work.[p]

Nonconforming work must follow the laboratory's cause analysis, risk assessment, and corrective action processes.[p]

[p]Based on IS17025:2017(E) Section 7.10 Nonconforming work

14. Document and Record Control

The laboratory must have a system to control and track the revisions of SOPs, policies, procedures, and external documents.[q] The system must be able to provide a complete list or be housed in an electronic document management system (EDMS, or LIMS) for all SOPs, policies, procedures, and external documents.

The laboratory must have procedures to control revisions, editing, and approval of SOPs and policies for use in the laboratory.[q] The most current controlled version of laboratory SOPs and documents must be available tall staff at their workspace for use. Each document must be approved prior to use in the laboratory by the laboratory management and/or quality personnel to ensure completeness, compliance, and technical correctness.[r]

The laboratory must have procedure(s) that are consistent with legal commitments, regulatory and client requirements. Records must include::

- Designation of authorized users for LIMS
- Retention
- Archival
- Disposition of records
- Storage
- Protection of documents and data including data archives. Back-up/retrieval and cloud-based data retention services.

The laboratory must have procedures to ensure that technical records are complete including all laboratory records and data allowing for an historical reconstruction of the data contained in the final report. This includes, but is not limited to the following:

- All raw data, bench sheets and sample receiving documentation
- Preparation and analysis information
- Instrumentation outputs
- Transfer files for LIMS input
- Data review checklists
- Quality assurance reviews
- Customer service completeness review
- Final report delivered to the customer
- Documentation of all reagents and reference materials indicating traceability, stability use and disposal
- Instrument maintenance, on-going and corrective.

[q]Based on IS17025:2017(E) Section 8.2 Management system documentation

[r]Based on IS17025:2017(E) Section 8.3 Control of management system documents

15. Corrective Action/Risk Analysis (CAP) Process[s]

The laboratory must have a written process describing how to address and record nonconforming work. Once nonconforming work is identified, the laboratory must take the following steps taddress the nonconforming work by applying a cause and risk analysis, and implementing corrective actions. The corrective action once implemented must be monitored or reviewed to ensure their efficacy.

- Address and correct the nonconforming work to control or initially correct the process,
- Evaluate to eliminate the nonconforming work through a review and analysis of data,
- Must have a procedure to perform and document a risk analysis for the nonconforming work
- Determine the cause or causes, as there is rarely a single cause
- Design and implement the corrective action, implement actions to eliminate the underlying cause or causes, and to prevent its recurrence.
- Review the corrective action to ensure its effectiveness on a periodic basis to be defined by the laboratory based on risk and severity. The laboratory must evaluate whether the nonconforming work and its corrective action warrants a change to the quality management system.

[s]Based on IS17025:2017(E) Section 8.7 Corrective actions

16. Internal Audits

Internal audits must be performed by the laboratory on a schedule to cover all laboratory activities on a laboratory determined periodic basis. [t] The laboratory should utilize both horizontal and vertical audit approaches across technical and management areas. The laboratory must retain records of internal audits including, not limited to; checklists, SOP, data review, and CAPA results. [t]

[t]Based on IS17025:2017(E) Section 8.8 Internal audits

17. Management Reviews[u]

Management Reviews are a tool tallow the laboratory to review laboratory activities, assess the effectiveness of the quality management system, CAPA, and external influences upon the laboratory activities. The management review must occur annually including, but not limited to the following areas of review:

- Internal and outside factors that are impactful to the laboratory business
- Changes to the scope, definition, and amount of incoming work
- Ensure Management goals are being met
- Policies and SOPs are relevant and appropriate
- Action items from previous management review meetings have been addressed
- Results from internal and external audits
- Status of CAPAs
- Feedback from customers and employees
- Status of complaints and trending
- Effectiveness of improvements and corrective actions
- Resource allocation (personnel, equipment, consumables, etc.)
- Need for changes to the quality management system

[u]Based on IS17025:2017(E) Section 8.9 Management review

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18. Analytical Technical Requirements for Chemical Analysis

For chemical analysis, the daily batch requirements for quality control must be able to demonstrate that the laboratory is able to produce data of known and documented quality that is fit for purpose for the end user or customer of the laboratory. These goals are documented in the analytical process through the calibration, batch quality control samples, and establishment and monitoring of laboratory generated control limits.

Prior to analysis of samples in a batch, calibration curves must be established for all target analytes; the calibrations must meet the criteria noted in the appendices. The lowest and highest points within a calibration curve represent the working range for the instrument. Accuracy is verified by performing an initial calibration verification (ICV) using a standard derived from a second source.

Where applicable, once calibration curves have been established and verified, a blank is analyzed to ensure that the instrument is free of contamination from the initial calibration or other sources. A continuing calibration verification (CCV) is performed as outlined in the individual analytical appendices to verify that the accuracy of the initial calibration is sustained. If a CCV fails, another CCV can be run immediately. If the second CCV fails, then the laboratory must perform corrective action which may include another initial calibration and instrument maintenance.

APPENDICES

19. Appendix A: Cannabinoid Analysis

Cannabinoids are found in (or structurally related to compounds found in) the plant Cannabis sativa L. Prior to the 2018 Farm Bill, cannabinoids as a class were largely considered to be illegal on a federal level; the Farm Bill authorized the production and sale of hemp, which was defined as "the plant Cannabis sativa L. and any part of that plant… with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis."[a] Individual states have further passed laws and developed regulations authorizing the sale of cannabinoids, including delta-9 tetrahydrocannabinol at concentrations higher than 0.3%.

The commonality between all cannabinoid types is their role in and interaction with the endocannabinoid system, more specifically the CB1 and CB2 receptors. Although these cannabinoid receptors can interact with several different classes of compounds, most state and federal regulations largely are limited to phytocannabinoids, which are cannabinoids produced directly by the Cannabis sativa L. plant. As a result, phytocannabinoids are therefore the focus of this appendix, but it should be noted that chemically modified cannabinoids have become increasingly relevant in the current regulatory environment, and will be discussed in more detail in a future whitepaper.

[a] H.R.2 - Agricultural Improvement Act of 2018.

Analytes:

Required Cannabinoid Analytes (chosen for their ubiquity in cannabis plant material and manufactured products)		
Compound	**Abbreviation**	**CAS Number**
Tetrahydrocannabinolic acid (A)	THCA-A	23978-85-0
Delta 9 – Tetrahydrocannabinol	Δ9-THC	1972-08-3
Delta 8 – Tetrahydrocannabinol	Δ8-THC	5957-75-5
Cannabinol	CBN	521-35-7
Cannabigerolic acid	CBGA	25555-57-1
Cannabigerol	CBG	25654-31-3
Cannabidiolic acid	CBDA	1244-58-2
Cannabidiol	CBD	13956-29-1
Additional Analytes (may be required by individual regulators)		
Compound	**Abbreviation**	**CAS Number**
(6aR,9R)-Delta 10 – Tetrahydrocannabinol	(6aR,9R)-Δ10-THC	95543-62-7
(6aR,9S)-Delta 10 – Tetrahydrocannabinol	(6aR,9S)-Δ10-THC	95588-87-7
(9R)-Delta 6a,10a – Tetrahydrocannabinol	(9R)-Δ(6a,10a)-THC	95720-01-7
(9S)-Delta 6a,10a – Tetrahydrocannabinol	(9S)-Δ(6a,10a)-THC	95720-02-8
Tetrahydrocannabivarinic acid	THCVA	39986-26-0
Tetrahydrocannabivarin	THCV	31262-37-0
Cannabichromenic acid	CBCA	185505-15-1
Cannabichromene	CBC	20675-51-8
Cannabichromevarin	CBCV	41408-19-9
Cannabinolic acid	CBNA	2808-39-1
Cannabidivarinic acid	CBDVA	24274-48-4
Cannabidivarin	CBDV	13956-29-1
Cannabicyclolic acid	CBLA	40524-99-0
Cannabicyclol	CBL	21366-63-2
Cannabicitran	CBT	31508-71-1
Any additional Cannabinoids, if labeled in a cannabis good		

Appendix A, Table 1: Required and Additional Cannabinoids

Calculation to determine Total Potential Cannabinoids in a sample:

- Total potential [cannabinoid] concentration (mg/g) = ([cannabinoid] acidic form concentration (mg/g) × 0.877) + [cannabinoid] concentration (mg/g) + ...)
  - where [Cannabinoid] = Any isomers of that specific cannabinoid (in the instance for THC, where there may be multiple isomers present)

Hemp Cannabinoid Analysis Protocol

- Per federal regulations hemp is defined to have a Total delta-9 THC concentration of < 0.3%. If the Total THC is greater than 0.3% then the material is considered marijuana, and is subject to additional legal restrictions and considerations for the lab in terms of handling and disposal.

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Technology/Equipment/Supplies

- Instrumentation recommended: LC-Diode Array Detection, LC-MS
- Methodologies recommended:
  - AOAC Official Method 2018.10 Cannabinoids in Cannabis sativa Dried Flowers and Oils Liquid Chromatography with UV Detection (First Action 2018)
  - AOAC Official Method 2018.11 Quantitation of Cannabinoids in Cannabis Dried Plant Materials, Concentrates, and Oils Liquid Chromatography–Diode Array Detection Technique with Optional Mass Spectrometric Detection (First Action 2018, Revised First Action 2020)
  - If the testing lab wishes to utilize or develop their own method for cannabinoid analysis, preferred analyte recoveries for different matrices can be found in the following SMPRs:
    - AOAC SMPR® 2019.003 Standard Method Performance Requirements (SMPRs®) for Quantitation of Cannabinoids in Plant Materials of Hemp (Low THC Varieties Cannabis sp.)
    - AOAC SMPR® 2017.001 Standard Method Performance Requirements(SMPRs) for Quantitation of Cannabinoids in Cannabis Concentrates
    - AOAC SMPR® 2017.019 Standard Method Performance Requirements (SMPRs®) for Quantitation of Cannabinoids in Edible Chocolate

Batch QC Requirements

- Prior to initiating a batch:
  - Initial Calibration Standards
  - Initial Calibration Verification (ICV) – must perform, at minimum, 2 ICV checks (high, low) at different levels in the calibration curve
- *If above ICVs pass, batches can be run following the sequence below
- Typical Batch QC/ Frequency/ Criteria:
- Batch size: 20 samples
  - Method Blank
  - CCV
  - LCS
  - Duplicate Sample(s) (can be a customer sample in the first set of 10)
  - Samples 1-10
  - CCV every 10 samples
  - Samples 11-20
  - CCV – closing

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Additional QC Recommendations

- Matrix-Specific Quality Control Sample – real, thoroughly characterized in-house sample that is run once a day to track accuracy and precision; should record values in a control chart for monitoring. The sample can also be used for inter-instrument comparisons. Ideal variance (measured by %RSD) should be less than 5%.

Batch Acceptance Criteria

(Note: Some LQC samples include both a "Warning" or "Failure" acceptance criteria. In general, the "Warning" criteria is still enough to deem the batch acceptable for reporting data but suggests a re-calibration or other corrective action prior to running the next batch. Acceptance criteria are more stringent than other analyses, both due to the lower variability provided by the detector type, as well as the expectations of the industry).

Laboratory Quality Control Sample	Frequency	Acceptance Criteria	Corrective Action
Method Blank	One per batch	Not to exceed LOQ	Perform a root cause analysis to determine the source of contamination. Re-prepare Method Blank and reanalyze entire analytical batch.
Laboratory Control Sample (LCS)	One per batch	Recoveries: Warning: 90-110% Failure: 95-115%	Failure: Re-prep LCS and reanalyze. If LCS fails again, determine if failure is a result of sample contamination or matrix effects, or due to improperly calibrated instruments. Remedy issue and reanalyze entire analytical batch.
Matrix Spike/Matrix Spike Duplicate Or Sample/ Sample Duplicate	One set per batch	RPD ≤ 30% for all analytes with concentrations ≥ LOQ	Reanalyze sample and associated matrix spike sample once.
Initial Calibration verification (ICV)	High-, Mid-, Low-points of the calibration curve with second source CRM	Recoveries: High: 95-105% Mid: 90-110% Low: 85-118%	If the recovery for any analyte is outside of the acceptance criteria, recalibrate the instrument. Perform a root cause analysis.
Continued Calibration Verification (CCV)	One per every 10 samples. All analytes in each CCV must meet the criteria.	Recoveries: Warning: 90-110% Failure: 95-115%	Reanalyze all samples that preceded the last CCV that met the acceptance criteria. If CCV samples continue to fail, then recalibrate the instrument.

[a]Adapted from the AOAC SMPR 2019.003 performance requirements for Low-THC hemp samples

Appendix A, Table 2: Batch QC Criteria for Cannabinoid Analysis

Calibration Criteria

- Basic calibration scheme
  - Recommend two separate calibration curves, separating acid/non-acid standards; acid standards will degrade more readily in protic solvents, and most non-acid standards come dissolved in a protic solvent (methanol).
    - Acid standards – should dilute with non-protic solvents only, example: acetonitrile
    - Non-acid standards – can dilute with either protic- or non-protic solvents, examples: acetonitrile, methanol
  - Dilution factors should be determined gravimetrically; accurate results for samples depend on an accurate calibration curve. Determining dilution factors for standards in g/g allows for higher accuracy and traceability.
  - Minimum number of calibration levels: 5 Levels with linear regression.
  - Calibration points concentrations are laboratory derived
  - Types of calibration:
    - Average Response Factor
    - Linear regression
    - Weighted linear regression (up t1/x)
    - Calibration criteria: $R^2 \geq 0.995$ and RSE < 25%
  - LOQ for analytes tested must be the lowest calibration level within the range of the calibration curve. If testing hemp samples, LOQ for d9-THC must be greater than 0.3% for all matrix types and preparations.

20. Appendix B: Microbial Analysis

Microbial contamination represents an important acute public health concern for cannabis, cannabis derived products and edible products containing cannabis. The testing for microbial contaminants is essential for ensuring public health and safety. The action limits and the organisms listed below are representative of a comprehensive testing program to ensure safer products. Due to the large and expanding acceptable performance tested methods that are acceptable for microbial detection in cannabis, a dedicated methods section was not included in this section. It is instead our recommendation to follow the methods listed on the performance tested methods that your lab utilizes. Any chosen deviations from those methods would be recommended to determine if a verification or validation is needed (see section 17 to help with the determination) and make sure that the appropriateness of the changes are in line with the lab's regulating bodies.

Analytes:

Recommended Minimum Achievable Limits (CFU/g)			
Microbes Tested	**Flower/ Inhalable Compound Concentrate Products**	**Cannabis-Infused Products**	**Cannabis Extract non-solvent & non-CO2**
Total Yeast and Mold	≤100,000	N/A inhalants only	≤1,000
Total Coliform Count	≤1,000	≤100	≤100
Total Aerobic	≤10^5	≤10^5	≤10^5
Bile-Tolerant Gram-Negative Bacteria	≤1,000	≤1,000	≤1,000
Shiga toxin-producing *E. coli* (STEC)	Not detected in 1 gram	Not detected in 1 gram	Not detected in 1 gram
Pathogenic *Salmonella* spp.	Not detected in 1 gram	Not detected in 1 gram	Not detected in 1 gram
Aspergillus flavus, fumigatus, niger & terreus	Not detected in 1 gram (Speciation not required but capability is recommended)	N/A inhalants only	Not detected in 1 gram (Speciation not required but capability is recommended)

Appendix B, Table 1: Recommended Action Limits for Microbial Contamination

Technology/Equipment/Supplies

- Instrumentation recommended: Plating/Petrifilm/Simplate, Plate Reader, Incubator, Thermocycler (Salmonella, STEC, Aspergillus)

Preparation

- Preparation Notes
  - Due to large variations in potential total batch sizes, specifically among cannabis growers, PCR sample weight requirements will be determined by percentage of the total sampled amount.
  - Proper incremental sampling is required in order to accurately analyze microbial loads. To ensure this is done a Sequestered Microbial Sample should be taken.
  - The sequestered microbial sample should fully represent the sample as a whole.
  - Sequestered microbial samples should be homogenized separately from the rest of the sample and using methods that will not impact the microbial load prior to testing. Cryogenic grinding or heat introduction may kill target microbes leading to potential false-negative results, leading to possible harm to the general public.
  - Most AOAC methods for PCR use 10-25g of sample for testing, however due to overall sample batch size this may create undue financial burden when a smaller sample weight may still adequately represent the whole of the batch. Results must include the sample weight used in the analysis (e.g. Negative in 5 g)
  - Verification of any methods using less than the validated amount will be required.
  - Following homogenization of the sequestered microbial sample, at least 10% of the total sample received, by weight, should be used as the PCR prep weight not exceeding 25g or subceeding 1g.
  - Plating will require 1.0 grams of sample
  - A phosphate buffer will be required for plating and an enrichment broth will be needed for PCR i.e. Tryptic Soy Broth or Potato Dextrose Broth

- If PCR is the detection method, it will require an enrichment and incubation of the sample, followed by DNA extraction, prior to running the PCR.
- For PCR detection methods it is recommended to have a "kill step" such as DNAse treatment prior to cell analysis to ensure that only viable DNA is amplified to prevent potential false positives.
- Plating will require dilution gradients for plating that accounts for action limits and accurate counting. Ideally a passing plate should have no more than 100 colonies growing on it.
    - For standard plating using traditional pour plate with agar, total plate count is recommended for plates to have 25-250 or 30-300 colonies to be considered a "countable" range. For total Yeast + Mold, the countable range is considered 10-150 colonies per plate.
    - For alternate methods such as Simplate and Petrifilm granted Performance Tested Method (PTM) status under AOAC for enumerations of yeasts and mold in dried cannabis flower, the countable range recommendations are as follows:
        - Simplate has a range of 10-7,380 for a single dilution
        - Petrifilm total plate count recommends 300 colonies or less
        - Petrifilm Yeast and Mold recommends a maximum 150 colonies
    - PCR batch size is determined by the plate capacity. Most standard thermocyclers have 96 wells, though other cyclers range from 36 t384 wells.
    - A batch or test run is defined as an uninterrupted series of analyses, generally 20 -30 samples including appropriate QC controls. The time limit between filtration of samples cannot exceed 30 mins with an overall batch time of 4 hrs.

Batch QC Requirements

- Plating
    - There should be an exposed plate tact as an environmental negative control to account for environmental contamination during the preparation period.
    - 1 Duplicate sample per batch RSD ≤30%
    - 1 Positive reference target organism spike of live microorganism per batch
    - 1 negative buffer blank per batch
- PCR
    - 1 duplicate sample per batch
    - 1 Positive reference target organism spike of live microorganism per prior to enrichment step per batch
    - 1 negative control blank broth prior to enrichment
    - Internal standards used for all samples to ensure proper amplification is performed by the thermocycler

Batch QC Acceptance Criteria

- Any failing positive or negative control QC will result in a complete batch retest
- If the duplicates fail but all other QC passes, then the sample can be reprepared by the original technician if that prep accurately passes one of the thresholds on one of the original samples the batch can be accepted and the original sample that matched the reprep is accepted
- For PCR if the Internal standard fails to amplify for a specific sample that sample must be prepared again and run in a different batch.

Calibration Criteria

- Basic calibration scheme
  - Plating does not require any calibration
  - Most thermocyclers are calibrated yearly following manufacturer's specification

21. Appendix C: Heavy Metals Analysis

The presence of toxic heavy metals is widespread in the environment. Some of the health issues associated with heavy metals are kidney disease, neuropathy, anemia, cancer, and developmental toxicity.1 Cannabis plants have an exceptional ability to bioaccumulate heavy metals from soil and thus, the impetus for testing cannabis products is even greater. The following appendix provides guidance for testing of heavy metals in cannabis and cannabis-containing matrices.

Analytes

Required elements for all cannabis and cannabis containing products:

Required Heavy Metal Analytes		
Element	**CAS #**	**Routine Achievable LOQ (ng/g)**
Arsenic	7440-38-2	50
Cadmium	7439-92-1	50
Lead	7440-43-9	125
Mercury	7439-97-6	60
Additional Analytes (may be required by individual regulators)		
Element	CAS #	
Antimony	7440-36-0	
Barium	7440-39-3	
Nickel	7440-02-0	
Total Chromium	7440-47-3	
Copper	7440-50-8	
Silver	7440-22-4	
Selenium	7782-49-2	
Zinc	7440-66-6	

Appendix C, Table 1: Analytes for heavy metal analysis

Technology/Equipment/Supplies

- Analytical Instrumentation Recommended: ICP-MS equipped with collision / reaction cell technology. Note: ICP-OES may be suitable in theory, however, the scarcity of literature precedence in the context of cannabis or food matrices precludes recommendation. Generally, ICP-MS is a much more sensitive technique. Mainstream ICP-OES instruments may not be capable of achieving reliable measurement of sub-ppm analyte concentrations, particularly in cannabis matrices.
- Sample preparation instrumentation recommended: Microwave Digestion System capable of reaching 210□.

Methodologies recommended:

- *AOAC Official Method 2021.03 Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products Inductively Coupled Plasma–Mass Spectrometry (First Action 2021)*
- If the testing lab wishes to utilize or develop their own method for heavy metals analysis, preferred analyte recoveries can be found in the following SMPR:
  - *AOAC SMPR® 2020.001 Standard Method Performance Requirements (SMPRs®) for Determination of Heavy Metals in a Variety of Cannabis and Cannabis-Derived Products*

Sample Preparation

- Sample Mass:
  - Minimum 0.5 g
- Digestion Technique (closed vessel system):
  - 0.5 g homogenized sample
  - Acids for digestion:
    - Nitric Acid (HNO_3): Concentrated (Ultrapure or equivalent)
    - Hydrochloric Acid (HCl): Concentrated (Ultrapure or equivalent)
      - Note: HCl used for Hg stabilization
  - Diluent for Sample Preparation:
    - 1% - 5% (v/v) HNO_3 / 0.5% (v/v) HCl solution in DI Water (Resistance > 18 MΩ•cm)
  - Rinse Blank:
    - 1% - 5% (v/v) HNO_3 / 0.5% HCl in DI Water (Resistance > 18 MΩ•cm)
- Additional notes on sample preparation and digestion:
  - Closed-vessel microwave digestion vs. hot plate digestion at ambient pressure:
    - Closed-vessel microwave digestion:
      - Preferred digestion method (easiest and most practical). A closed-vessel digestion system significantly reduces loss of nitric acid. Increased vessel pressure increases the nitric acid boiling point; therefore, higher digestion temperatures can be reached. Higher temperatures (up to 210 °C) are often needed for complete digestion of complex matrices. Time required for digestion is approximately 1 hour, depending on the microwave method.

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- Hot plate digestion (not recommended):
  - The boiling point of nitric acid at ambient pressure is ~181 °C. Often, plant materials and concentrates require temperatures > 181 °C for full digestion. The digestion process may take several hours (i.e., 3-5 hours). During this time, the operator must replenish the nitric acid that boils out of the mixture. The possibility of contamination is greater with an open container. Additionally, mercury can potentially evaporate out of the solution.

- Predigestion recommended for extracts, distillates, isolates, etc. A 15-minute predigestion to initiate the breakdown of hydrocarbons. Use caution when digesting samples containing alcohol (e.g. cannabis ethanol extracts) as they will rapidly increase in volume and can produce a violent reaction.
- Glass vials - may contain lead. Acid wash recommended before use.
- Closed vessels - be vigilant of ruptured caps; may hinder reaching higher digestion temperatures.
- Hydrogen peroxide (H_2O_2) - using a small volume of hydrogen peroxide may be useful for achieving full digestion of difficult matrices (e.g. concentrates), however it is typically not necessary in most cases. Addition of H2O2 leads to regeneration of nitric acid, and thus helps in sustaining the digestion process. Note: it dilutes the acid strength; denatures into water at high temperatures (~ 150 °C).

Batch QC Requirements :

- Calibration:
  - Initial Calibration Standards
  - Initial Calibration Verification (ICV) - either an alternate lot or alternate product containing the same analytes, or an alternate manufacturer. If the ICV passes (according to guidelines in Table 1), continue the batch sequence described below.
- Batch size: ≤ 20 samples
  - Reagent Blank
  - Continuing Calibration Verification (CCV)
  - Method Blank
  - Laboratory Control Sample (LCS)
  - Matrix Spike (not mandatory; informative)
  - Duplicate of the LCS or duplicate of matrix spike (for precision)
  - Samples 1-10
  - CCV every 10 samples
  - Samples 11-20
  - CCV – closing

Batch QC Acceptance Criteria

Laboratory Control Sample	Frequency	Acceptance Criteria	Corrective Action
Method Blank	One per digestion batch to ensure no contaminants in vessels	Not to exceed LOQ	Failure: Reanalyze sample; if the sample fail again, perform a root cause analysis to determine the source of contamination, reprep and reanalyze the entire analytical batch. .
Laboratory Control Sample (LCS)	One per batch	Recoveries: Warning: 90-110% Failure: 80-115%	Failure: Re-prep and reanalyze entire analytical batch, or if necessary, re-run initial cal curve
Matrix Spike	One per batch Refer to AOAC SMPR 2021.001, run a blank matrix, prior to spiking	No accuracy requirement (informative)	N/A
Laboratory Control Sample Duplicate or Matrix Spike Duplicate	One or the other; purpose is to assess batch precision.	RPD < 30%	Failure: Reanalyze samples; if the RPD is still > 30%, reprep and reanalyze all samples in the analytical batch.
Initial Calibration verification (ICV)	Mid-point on the calibration curve with second source standard; to be run immediately after the calibration curve.	% Recovery must be between 80-115%a:	Failure: Reanalyze ICV one time. If it fails again, determine source of failure, re-prep and reanalyze ICV and/or calibration curve, if necessary.
Continued Calibration Verification (CCV)	Each set of 10 or portion of 10 samples must be bracketed by CCVs. Each CCV must meet the criteria. Minimum of three CCVs per batch of 20 (or two per batch of 10).	% Recovery must be between 80-115%[a]:	Failure: Reanalyze all samples that are either followed by or preceded by failing CCVs. Two consecutive CCV failures should be followed by troubleshooting and re-running the initial cal curve and all samples in the analytical sequence

[a]Refer to AOAC SMPR® 2020.001

Appendix C, Table 2: Batch Acceptance Criteria for Heavy Metal Analysis

Calibration Criteria

- Basic Calibration Scheme
  - Number of Calibration Levels: minimum 5
    - Prepared weekly or as needed to maintain QCs
  - Calibration Concentrations:
    - Determined by laboratory based on concentration of analytes typically found in samples
    - Calibration Blank: 1 - 5% HNO3 / 0.5% HCl in DI Water (Resistivity > 18 MΩ⍰cm)

- Prepare blank solutions the same day as analysis
- Regression: Linear or weighted linear (1/x)
- R2 ≥ 0.995; RSE < 25%
- LOQ for analytes tested must be either the lowest calibration level within the range of the calibration curve or at a concentration that achieves a S/N > 10 within the calibration curve.

- NOTE: It is suggested/required in some states that a CRM/SRM be included as a sample for validation.

Calculations

Note: These can be automatically performed in most software platforms

- Concentration (ppb, ng/g) = S × DF × (*M*/*m*)
  - S: concentration of analyte in analytical solution (ng/g)
  - *M*: mass (g) of analytical solution
  - *m*: mass of analytical portion (g)
  - DF: dilution factor (=1 if analytical solution is not diluted)
- Spike Recovery (%) = $[(C_{x+s}-C_x) / (C_sM_s/M_x)] \times 100$
  - C_{x+s}: concentration determined in spiked sample (ng/g)
  - C_x: concentration determined in unspiked sample (ng/g)
  - C_s: concentration of spiking solution (ng/g)
  - M_s: mass of spiking solution added tan analytical portion (g)
  - M_x: mass of analytical portion (g)
- Interference Corrections:

  Isobaric interferences can interfere with the analyte signal. Interference from polyatomic and doubly-charged isobaric species can be sufficiently mitigated (although not always fully) by operating in KED mode (Kinetic Energy Discrimination) using He gas in a collision cell. Elemental isobaric interferences and residual polyatomic or doubly charged isobaric interferences can be mathematically corrected for using interference correction equations that are well-known in literature.[2,3]

  - Interference correction equation for ^{111}Cd: Corrects for residual MoO interference
    - Mc(111) = M(111) - M(108) × 1.18 + M(106) × 0.712
  - Interference correction equation for ^{115}In: Corrects for ^{115}Sn interference
    - Mc(115) = M(115) - M(118) × 0.0149
  - Interference correction equation for ^{75}As:
    - Correcting for $^{150}Sm^{2+}$ and $^{150}Nd^{2+}$
      - Mc(75) = M(75) - M(72.5) × 0.6747 - M(73.5) × 0.4923

- Correcting for $^{75}ArCl^{+}$ and ^{77}Se
  - $Mc(75) = M(75) - M(77) \times 3.13 + M(82) \times 2.73$
- Pb Isotopes: Summation of main Pb isotopes at m/z 206, 207 & 208
  - $Mc(208) = M(206) + M(207) + M(208)$

Reporting

- Ultimately, analyte concentrations should be converted / reported in µg/g or ng/g of sample

References

1. Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B. B.; Beeregowda, K. N.; "Toxicity, mechanism and health effects of some heavy metals," **2014**, 7, 60-72.
2. May, T. W.; Wiedmeyer, R. H. "A Table of Polyatomic Interferences in ICP-MS"; *Atomic Spectroscopy*, **1998**, 19, 150-155.
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22. Appendix D: Pesticides and Mycotoxin Analysis

The following appendix provides guidance for analysis of pesticides and mycotoxins in cannabis and cannabis-derived products. The pesticide analytes to be considered are listed in Table 1; the list was adapted from the document AOAC SMPR 2018.011. The recommended target LOQs described in the AOAC document were deemed to be impractically low in the context of non-hemp cannabis plant material, therefore, target LOQs were revised based on a survey of achievable LOQs collected from several testing laboratories. Pesticide analysis can be divided between LC-MS/MS and GC-MS/MS methods. The mycotoxin analytes to be considered are listed in Table 2. Typically, LC-MS/MS is the preferred method for mycotoxin analysis. Often, pesticides and mycotoxins can be analyzed using the same analytical method, therefore, the guidance is combined in this appendix.

Analytes

Compound	CAS #	Routine Achievable LOQ (ug/g)
Abamectin	71751-41-2	0.1
Acephate	30560-19-1	0.06
Acequinocyl	57960-19-7	0.1
Acetamiprid	135410-20-7	0.06
Aldicarb	116-06-3	0.075
Allethrin	584-79-2	0.2
Ancymidol	12771-68-5	0.05
Azadirachtin	108168-76-9	1.0
Azoxystrobin	131860-33-8	0.06
Benzovindiflupyr	1072957-71-1	0.05
Bifenazate	149877-41-8	0.06

Compound	CAS #	Routine Achievable LOQ (ug/g)
Bifenthrin	82657-04-3	0.1
Boscalid	188425-85-6	0.06
Buprofezin	69327-76-0	0.05
Captan	133-06-2	0.6
Carbaryl	63-25-2	0.1
Carbofuran	1563-66-2	0.06
Chlorantraniliprole	500008-45-7	0.1
Chlordane	57-74-9	0.075
Chlorfenapyr	122453-73-0	0.0875
Chlormequat chloride	999-81-5	0.1
Chlorpyrifos	2921-88-2	0.06
Clofentezine	74115-24-5	0.06
Clothianidin	21088-92-5	0.05
Coumaphos	56-72-4	0.06
Cyantraniliprole	736994-63-1	0.02
Cyfluthrin	68359-37-5	0.3
Cypermethrin	52315-07-8	0.3
Cyprodinil	121552-61-2	0.05
Daminozide	1596-84-5	0.1
Deltamethrin	52918-63-5	0.3
Diazinon	333-41-5	0.06
Dichlorvos	62-73-7	0.075
Dimethoate	60-51-5	0.06
Dimethomorph	110488-70-5	1.0
Dinotefuran	165252-70-0	0.05
Dodemorph	1593-77-7	0.05
Endosulfan 1 (alpha)	959-98-8	0.05
Endosulfan II (beta)	33213-65-9	0.15
Endosulfan sulfate	1031-07-8	0.075

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Compound	CAS #	Routine Achievable LOQ (ug/g)
Ethephon	16672-87-0	1.0
Ethoprophos	13194-48-4	0.06
Etofenprox	80844-07-1	0.06
Etoxazole	153233-91-1	0.06
Etridiazole (Terrazole)	2593-15-9	0.03
Fenhexamid	126833-17-8	0.1
Fenoxycarb	79127-80-3	0.06
Fenpyroximate (mix of isomers)	111812-58-9	0.06
Fensulfothion	115-90-2	0.02
Fenthion	55-38-9	0.02
Fenvalerate (Sanmarton)	51630-58-1	0.1
Fipronil	120068-37-3	0.06
Flonicamid	158062-67-0	0.06
Fludioxonil	131341-86-1	0.06
Fluopyram	658066-35-4	0.02
Flurprimidol	56425-91-3	0.01
hexythiazox	78587-05-0	0.06
Imazalil	35554-44-0	0.06
Imidacloprid	138261-41-3	0.06
Iprodione	36734-19-7	0.5
Kinoprene	37882-31-8	0.5
Kresoxim-methyl	143390-89-0	0.06
Malathion	121-75-5	0.06
Metalaxyl	57837-19-1	0.1
Methiocarb	2032-65-7	0.06
Methomyl	16752-77-5	0.2
Methoprene	40596-69-8	2.0
Methyl Parathion	298-00-0	0.06
Mevinphos	7786-34-7	0.06

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Compound	CAS #	Routine Achievable LOQ (ug/g)
MGK-264	113-8-4	0.1
Myclobutanil	88671-89-0	0.06
Naled (Systhane)(Dibrom)	300-76-5	0.075
Novaluron	116714-46-6	0.05
Oxamyl	23135-22-0	0.25
Paclobutrazol	76738-62-0	0.06
Pentachloronitrobenzene (Quintozene)	82-68-8	0.1
Permethrin (mix of isomers)	52645-53-1	0.3
Phenothrin (d-phenothrin)	26002-80-2	0.05
Phosmet (Imidan)	732-11-6	0.06
Phosmet (oxon)	3735-33-9	0.1
Piperonyl butoxide	51-03-6	0.5
Pirimicarb	23103-98-2	0.02
Prallethrin (mix of isomers)	23031-36-9	0.1
Propiconazole (tilt)	60207-90-1	0.06
Propoxur (Baygon)	114-26-1	0.06
Pyraclostrobin	175013-18-0	0.02
Pyrethrin (mix of isomers)	8003-34-7	0.3
Pyridaben	96489-71-3	0.06
Resmethrin	10453-86-8	0.1
Spinetoram	187166-40-1	0.06
Spinosad (mixture of A and D)	168316-95-8	0.06
Spirodiclofen	148477-71-8	0.1
Spiromesifen	283594-90-1	0.1
Spirotetramat	203313-25-1	0.06
Spiroxamine	118134-30-8	0.06
Tebuconazole	107534-96-3	0.06
Tebufenozide	112410-23-8	0.02
Teflubenzuron	83121-18-0	0.05

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Compound	CAS #	Routine Achievable LOQ (ug/g)
Tetrachlorvinphos	961-11-5	0.02
Tetramethrin	7696-12-0	0.1
Thiacloprid	111988-49-9	0.06
Thiamethoxam	153719-23-4	0.25
Thiophanate-methyl	23564-05-8	0.05
Trifloxystrobin	141517-21-7	0.06

adopted from AOAC SMPR 2018.011

Appendix D, Table 1: Pesticide Analytes to be Considered

Analyte	CAS #	Routine Achievable LOQ (ug/g)
Aflatoxin B1	1162-65-8	0.005
Aflatoxin B2	7220-81-7	0.005
Aflatoxin G1	1165-39-5	0.005
Aflatoxin G2	7241-98-7	0.005
Ochratoxin A	303-47-9	0.02

Appendix D, Table 2: Mycotoxin Analytes to be Considered

- Concentration ranges for each analyte
  - Range of concentrations that produces a linear calibration curve
  - Pesticide and mycotoxin concentrations typically found in samples that the laboratory has received should be considered when defining the upper limit of the calibration curve.

Technology/Equipment/Supplies

- Instrumentation recommended:
  - Both LC-MS/MS and GC-MS/MS instruments at a minimum.
- Instrument peripherals recommended:
  - Columns: biphenyl, polar C18, or comparable.
  - MS sources for LC-MS/MS: Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI)

Preparation

- Preparation Notes
  - Sample amount: no less than 0.5 gram, provided that the sample is homogenized thoroughly (particularly biomass, edibles).
  - Solvents/Reagents
    - LC-MS grade solvent (refer to standardized methods)
    - Strongly suggest use of internal standards (isotopically labeled), particularly for analytes that are prone to degradation or issues with extraction from matrix.
    - Appropriate additives, if needed, promote stabilizing analytes that are prone to degradation.
  - Preparation technique
    - Homogenization
      - Blending using a food processor or similar apparatus
      - Stirring (extracts, oils, tinctures, etc.)
      - Cryogrinding
    - Extractions Types
      - Solid-liquid extraction
      - QuEChERS
      - SPE
  - Final extract
    - Final Volume - dilution volume determined by individual labs
    - Final Reagents - determined by individual labs

Batch QC Requirements

- Calibration:
  - Initial Calibration Standards
  - Initial Calibration Verification (ICV) - if the ICV passes (according to guidelines in Table 3), continue the batch sequence described below.
- Batch size: ≤ 20 samples
  - Continuing Calibration Verification (CCV)
  - Reagent Blank
  - Method Blank
  - Laboratory Control Sample (LCS; Spiked Method Blank)

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- MS/MSD (for precision and informative)
  - sample duplicate if MS/MSD are not available
- Samples 1-10
- CCV every 10 samples
- Samples 11-20
- CCV-closing

- Batch acceptance criteria is described in Table 3.

- Additional Considerations:
  - It is recommended that each analyte/internal standard peak includes at least one qualifying ion to confirm identity.
  - *Analytes with multiple isomers:* Several analytes in Table 1 are composed of multiple isomers or derivatives that are represented by a single CAS number (e.g. chlordane, spinosad, pyrethrins, etc.). Often there is no information available about concentrations of individual components on the certificate of analysis. For example, a technical grade mixture of chlordane may contain >100 derivatives that make up the 1000 ug/mL certified concentration. Most compounds with multiple isomers can be categorized into two groups: 1) If individual concentrations of each isomer or derivative are clearly listed on the certificate of analysis, they should be integrated, calibrated, and quantified as separate peaks and then summed to produce the reportable concentration. 2) If isomer/derivative peaks are overlapping or very close together (i.e., within the same scan window) in the chromatographic profile, they should be integrated, calibrated, and quantified together as one peak. In the case of complex mixtures of isomers (e.g. chlordane), it is not feasible to try to quantify and sum all isomers in the mixture. Instead, the recommended course of action is to quantify at least two of the most prominent isomers/derivatives using the certified concentration and then average the measurements to represent the total analyte concentration. For example, alpha- and gamma-chlordane, the two most prominent isomers in technical grade chlordane, can be individually quantified and then averaged to represent the concentration of the >100 derivatives present in the mixture.

Batch QC Acceptance Criteria

Laboratory Quality Control Sample	Frequency and Level	Acceptance Criteria	Corrective Action
Method Blank (Definition: a pre-tested matrix that is free of analytes, prepared in the same way as a typical sample).	1 per batch	Not to exceed LOQ	**Failure:** Perform a root cause analysis to determine the source of contamination. If the reagent(s) is not contaminated, reanalyze entire analytical batch. If reagent(s) is contaminated, re-prepare samples with uncontaminated reagent(s) and reanalyze entire analytical batch.
Laboratory Control Sample (LCS) (Definition: a pre-tested matrix that is free of analytes - same as method blank - spiked with a known concentration of all analytes, and prepared in exactly the same way as a typical sample)	2 LCS per batch. One low level LCS (at or near action limit) to determine if sensitivity is maintained in batch; one mid-level LCS.	Recovery 70% t130%	**Failure:** Re-analyze the LCS. If the LCS fails again, re-prepare samples and reanalyze or re-run initial cal curve.
Matrix Spike/Matrix Spike Duplicate (a sample within the batch spiked with a known level of analytes)	2 per batch (1 MS, 1 MSD) Spike should be mid-level	- relative percent difference (RPD) must be <30% - no accuracy requirement (for informational purposes, such as the evaluation of matrix effects)	**Failure:** Reanalyze sample and associated matrix spike sample once. If RPD is still not acceptable, re-prepare samples and reanalyze
Initial Calibration verification (ICV) - a second source CRM	- Midpoint of the calibration curve - One ICV run directly after calibration curve	Recovery 70% t130%	**Failure:** Reanalyze the ICV once, if it fails again, re-prepare ICV. If the re-prepared ICV fails, re-prepare and/or re-run calibration curve as necessary.
Continued Calibration Verification (CCV)	Each set of 10 or portion of 10 samples must be bracketed by CCVs. Each CCV must meet the criteria. **Minimum of three CCVs per batch of 20 (or two per batch of 10)**	Recovery 70% t130%	**Failure:** Reanalyze all samples that are either followed by or preceded by failing CCVs. Two consecutive CCV failures should be followed by troubleshooting and re-running the initial cal curve and all samples in the analytical sequence

Appendix D, Table 1: QC Acceptance Criteria for Pesticide Batches

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Calibration Criteria

- Basic calibration scheme
  - Number of calibration levels: minimum six-point calibration for quadratic curves, minimum five-point calibration for linear curves.
  - Type of calibration - Linear, weighted linear, or quadratic regression
  - Calibration concentrations - LOQ determined as 50% of the action limit; upper range should be dictated by typical concentrations seen in samples by lab.
  - Calibration criteria - RSE < 30%. R2 value for curves minimum 0.99.
  - Calibration frequency - instrument should be calibrated as often as necessary; dependent on the results of the ICV, CCV, and/or QC samples. If significant drift is detected, then consider recalibration.
- ICV
  - Should be run immediately after the instrument calibration
  - Criteria 70-130 % recovery

23. Appendix E: Moisture Content Analysis

Moisture content and water activity are two analytical procedures to measure the amount of water in a sample. In cannabis and hemp labs, the two procedures are performed for distinct and different reasons. Moisture content is commonly used to determine a dry weight correction factor, while water activity is useful mainly in determining the ability of microorganisms to grow on the sample.

Moisture Content is the measure of the quantity of water present in a sample, expressed as a percentage by weight of the total sample. The historical importance of the measurement of moisture content in the cannabis and hemp spaces is to provide a correction factor for the amount of water in the plant material as a way to normalize the measurement of other cannabinoids, particularly delta-9 tetrahydrocannabinol (d9-THC). Raw hemp material typically contains 70-80% water by weight when removed from the field and is then dried to under approximately 15% moisture prior to performing analysis.

The 2018 Farm Bill defines hemp as "the plant Cannabis sativa L. and any part of that plant, including the seeds thereof and all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers, whether growing or not, with a delta-9 tetrahydrocannabinol concentration of not more than 0.3 percent on a dry weight basis." In response, the USDA has established guidelines for the analytical testing of hemp that require the concentration of delta-9 tetrahydrocannabinol to be determined and reported on a dry weight basis. The final reported result is corrected for moisture to report on the dry weight basis.

Moisture is measured either through gravimetric means (either Loss on Drying, which involves cycles of heating and weighing a sample to measure the loss of water, or a Moisture Analyzer, which is a balance with a built-in heater that applies heat directly to the sample to vaporize water and measures the change in mass) or through chemical means using Karl Fischer, the determination of free water by measuring the oxidation reaction of iodine and sulfur dioxide in the presence of water.

Technology/Equipment/Supplies

- Instrumentation recommended:
  - Oven - for Loss on Drying
  - Moisture Analyzer
  - Karl Fischer Titrator
- Instrumentation must be qualified and calibrated prior to use

Moisture Methodologies/References recommended:

- AOAC 930.15 Moisture in Animal Feed
- AOAC 966.02 Loss on Drying (Moisture) in Tobacco
- USP <731> Loss on Drying
- USP <921> Water Determination
- The method of analysis for moisture, regardless of technique, must be validated to ensure the accuracy and precision of the results. The impact on the final reported results should also be evaluated, such that the moisture result does not negatively influence the dry weight result.

Batch QC Requirements

(Where applicable, in some cases based on instrumentation used a particular QC requirement may not apply) See Tables 1 through Table 3 for instrument specific batch QC Requirements.

- Recommended max batch size 20 samples

Additional QC Requirements:

The laboratory should periodically participate in a PT program.

Batch QC Acceptance Criteria

Laboratory Control Sample	Method	Frequency	Acceptance Criteria	Corrective Action
Laboratory Control Sample (LCS)	Spike a sample with water to determine recovery (e.g. Cellulose)	Once per batch	90-110 % Recovery	Verify the calibration of the balance. Out of specification investigation.
Duplicate Sample	NA	One set of duplicates per batch	< 10 % Relative Percent Difference	Homogenize sample again sample and reanalyze.
Continued Calibration Verification (CCV)	Verify the Balance using a Calibrated Weight Set	Daily	< 0.1 % difference from assigned mass	If balance calibration is invalidated remove equipment from service, and calibrate using an IScertified provider.

Appendix E, Table 1: QC Acceptance Criteria for the Loss on Drying/Moisture Analyzer Technique

Laboratory Control Sample	Method	Frequency	Acceptance Criteria	Corrective Action
Instrument Standardization/ Titer Determination	Perform based on manufacturers recommendation or defined procedure.	Daily	NA	Variability in determined titer values could be indicative of poor analytical technique or instrument performance. Perform root cause analysis to determine source of error.
Method Blank	Determine impact of potential environmental moisture by titrating empty vessel using the same solvents used under test.	Once per batch	< LOQ	Perform a root cause analysis to determine the source of contamination
Duplicate Sample	NA	One set of duplicates per batch	< 10 % Relative Percent Difference	Homogenize sample again sample and reanalyze.
Continued Calibration Verification (CCV)	Perform a water standard check.	Every 10 samples	Within the stated criteria of the water standard used.	Perform a root cause analysis to determine the assignable cause (e.g. room humidity or sample homogeneity). Rerun standard, after consecutive failures reanalyze samples since last passing CCV.

Appendix E, Table 2: QC Acceptance Criteria for the Karl Fischer Technique

24. Appendix F: Residual Solvents Analysis

Solvents are volatile chemicals that are often used in the extraction of cannabinoids and in the processing of cannabis products. Residual solvents may remain if proper techniques are not used to remove them completely. Residual solvents analysis deals with the identification and quantitation of these remaining compounds in finished and unfinished cannabis products.

Residual Solvents & Processing Chemicals Analytes:

Individual states and regulatory bodies have significant differences in the analyte lists and action limits for residual solvents and processing chemicals. Tables 1-3 below present a comprehensive list of analytes from USP <467> (developed for pharmaceutical products); along with recommended action limits (most action limits from USP <467>, unless indicated otherwise). Analytes that are commonly used in cannabis and hemp are selected in bold, and should be considered as a minimum list of analytes to include as required analytes to screen for.

It is important to recognize USP<467> was developed using GC-FID as the analytical technique for the analysis of pharmaceutical raw material ingredients and not for plant material. The USP <467> method also requires a secondary column confirmation since an FID is a non-specific detector.

Since cannabis plant material and derived products are considered complex matrices, using an FID for detection most likely will not be sufficient to reach the concentration limits in the tables below. For these reasons, GC-MS detection is recommended to achieve the additional sensitivity and specificity required to meet these limits for cannabis plant and product matrices and also eliminates the need for a secondary column confirmation of results.

Note: The analytes provided in Tables 1-3 include routine achievable LOQs provided from laboratories participating in the development of this document; analytes in these tables that are not tested by any participating laboratory were left without LOQ recommendations.

Solvent	CAS Number	USP Health Concentration Limit (ppm)[a]	Routine Achievable LOQs (ppm)	Concern
Benzene	71-43-2	2	0.6	Carcinogen
Carbon Tetrachloride	56-23-5	4	-	Toxic and environmental hazard
1,2-Dichloroethane	107-06-2	5	1	Toxic
1,1-Dichloroethene	75-35-4	8	4	Toxic
Ethylene Oxide	75-21-8	1	2.5	Carcinogen, neurological impairment
1,1,1-Trichloroethane	71-55-6	1500[b]	-	Environmental hazard

[a] From USP 467 Interim Revision Announcement (unless otherwise indicated) .
[b] OSHA Standard 29 CFR 1910.1047
Appendix F, Table 1: Residual Solvents Class 1 - Solvents to be Avoided

Solvent	CAS Number	USP Health Conc Limit (ppm)[a]	Routine Achievable LOQs (ppm)	Solvent	CAS Number	USP Health Conc Limit (ppm)[a]	Routine Achievable LOQs (ppm)
Acetonitrile	75-05-8	410	200	2-Methoxyethanol	109-86-4	50	-
Chlorobenzene	108-90-7	360	-	Methylbutylketone	591-78-6	50	-
Chloroform	67-66-3	60	1	Methylcyclohexane	108-87-2	1180	-
Cumene	98-82-8	70	-	Methylene chloride	75-09-2	600	62.5
Cyclohexane	110-82-7	3880	-	Methylisobutylketone	108-10-1	4500	-
1,2-Dichloroethene	156-59-2	1870	-	N-Methylpyrrolidone	872-50-4	530	-
1,2-Dimethoxyethane	110-71-4	100	-	Nitromethane	75-52-5	50	-
N,N-Dimethylacetamide	127-19-5	1090	-	Pyridine	110-86-1	200	-
N,N-Dimethylformamide	68-12-2	880	-	Sulfolane	126-33-0	160	-

Solvent	CAS Number	USP Health Conc Limit (ppm)[a]	Routine Achievable LOQs (ppm)	Solvent	CAS Number	USP Health Conc Limit (ppm)[a]	Routine Achievable LOQs (ppm)
1,4-Dioxane	123-91-1	380	-	Tetrahydrofuran	109-99-9	720	-
2-Ethoxyethanol	110-80-5	160	-	Tetralin	119-64-2	100	-
Ethylene glycol	107-21-1	620	-	Toluene	108-88-3	890	200
Formamide	75-12-7	220	-	Trichloroethylene	79-01-6	80	0.5
Hexane[b]	110-54-3	290	200	Xylene	1330-20-7	2170	200
Methanol	67-56-1	3000	500	Ethylbenzene[c,d]	100-41-4	100	7

[a] From USP 467 Interim Revision Announcement (unless otherwise indicated). [b] Sum of isomers.
[c] May be combined with xylenes. [d] OSHA Standard 1910.1000

Appendix F, Table 2: Residual Solvents Class 2 - Solvents to be Limited

Solvent	CAS Number	USP Health Conc Limit (ppm)[a]	Routine Achievable LOQ	Solvent	CAS Number	USP Health Conc Limit (ppm)[a]	Routine Achievable LOQ
Acetic acid	64-19-7	5000	-	Heptane	142-82-5	5000	700
Acetone	67-64-1	5000	700	Isobutyl acetate	110-19-0	5000	-
Anisole	100-66-3	5000	-	Isopropyl acetate	108-21-4	5000	-
1-Butanol	71-36-3	5000	-	Methyl acetate	79-20-9	5000	-
2-Butanol	78-92-2	5000	-	3-methyl-1-butanol	123-51-3	5000	-
Butyl acetate	123-86-4	5000	-	Methylethylketone	78-93-3	5000	-
tert-Butylmethyl ether	1634-04-4	5000	-	2-Methyl-1-propanol	78-83-1	5000	-
Dimethyl sulfoxide	67-68-5	5000	-	Pentane[b]	109-66-0	5000	200
Ethanol	64-17-5	5000	700	1-Pentanol	71-41-0	5000	-
Ethyl acetate	141-78-6	5000	200	1-Propanol	71-23-8	5000	-
Ethyl ether	60-29-7	5000	700	2-Propanol	67-63-0	5000	700
Ethyl formate	109-94-4	5000	-	Propyl acetate	109-60-4	5000	-
Formic acid	64-18-6	5000	-	Triethylamine	121-44-8	5000	-
Propane[c,d]	74-98-6	5000	500	Butane[b,d]	106-97-8	5000	200

[a] From USP 467 Interim Revision Announcement (unless otherwise indicated). [b] Sum of isomers.
[c] OSHA Standard 1910.1000. [d] NIOSH REL (recommended exposure limits).

Appendix F, Table 3: Residual Solvents Class 3 - Solvents with Low Toxic Potential

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Technology/Equipment/Supplies

- Instrumentation recommended:
  - Headspace GC-MS (preferred - headspace autosampling leads to lower matrix interferences, MS more definitively identifies the compounds of interest)
  - Liquid Injection GC-MS
- Equipment recommended:
  - Mininert valves - valves that allow for sampling of standards with a gastight syringe, yet create an airtight seal to avoid evaporation of volatile solvents
  - Vials that fit Mininert caps - should match closely with target volumes for standards, to minimize headspace in standard vial
  - Headspace vials - 10 mL: can help with sensitivity, 20 mL: may help avoid evaporation after addition of solution to vial
- Methodologies recommended:
  - **USP 467 Residual Solvents**
- If the testing lab wishes to utilize or develop their own method for Residual solvent analysis, that method must be validated in each matrix type (concentrate, flower, tincture, edible, topical, etc) that will be tested in the laboratory to ensure the accuracy and precision of the results.

Additional Laboratory Considerations:

- The extraction/dilution solvent chosen for preparation of any standards or samples must follow two rules:
  - All analytes must be soluble in the extraction solvent
  - The extraction solvent must not be included on the analyte list itself. Common solvents for residual solvent analysis are N,N-dimethylacetamide (DMA or DMAc), dimethylsulfoxide (DMSO), triacetin, and trimethylbenzene
- The laboratory should be set up to avoid contamination by other solvents used in the preparation of laboratory samples. Examples can include:
  - Storing and using laboratory solvents in a fume hood
  - Preparing residual solvent samples in a separate fume hood or separate area of the building
- Background levels of contamination from laboratory solvents must be well-controlled and well below the action limit for each solvent. The determination of the LOD/LOQ for each analyte must take the background levels of the analyte into consideration; if the LODs are set below the background levels for each analyte, the laboratory may be reporting results for those analytes that are not representative of the sample. This can be achieved by following the protocol described in EPA 40 CFR 136 Appendix B, which involves a statistical analysis of variance using at least 7 samples spiked with analytes at levels near an estimated detection limit.

Batch QC Requirements

- Prior to starting an analytical batch:
  - Initial Calibration Standards
  - Initial Calibration Verification (ICV) – *If ICV passes, BEGIN BATCH SEQUENCE below

Typical Batch QC/ Frequency/ Criteria:

- Batch size: 20 samples
  - Instrument Blank (aka Reagent Blank)
  - Method Blank
  - CCV
  - LCS (Spiked Method Blank)
  - Duplicate Sample(s)
  - Samples 1-10
  - CCV every 10 samples
  - Samples 11-20
  - CCV – closing

Batch QC Acceptance Criteria

Laboratory Control Sample	Frequency	Acceptance Criteria	Corrective Action
Method Blank	One per batch	Not to exceed LOQ	**Failure:** Perform a root cause analysis to determine the source of contamination and remove/remedy it. *Common sources of contamination: 1) mobile phase waste from LC analysis. 2) solvents used in preparation of other analyses, 3) other samples with high levels of solvent 4) carryover from previous injection* Reanalyze a new Method/Reagent Blank with entire analytical batch. If solvent for Reagent Blank is contaminated, re-prep and reanalyze entire analytical batch using a different solvent source.
Laboratory Control Sample (LCS)	One per batch.	Recoveries: 70-130% (analytes with BP<0°C - propane, butane, isobutane - should be exempted from recovery requirements provided the analytes pass in the CCV)	**Failure:** Re-prep LCS and reanalyze. If LCS fails again, determine if failure is a result of sample contamination or matrix effects, or due to improperly calibrated instruments. Remedy issue and reanalyze entire analytical batch.
Laboratory Replicate Sample	One per batch	< 20 % RPD for all analytes with concentrations >LOQ	Reanalyze the sample and associated matrix spike sample once to verify the matrix impact. **Failure:** Re-prep samples and reanalyze

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Laboratory Control Sample	Frequency	Acceptance Criteria	Corrective Action
Initial Calibration verification (ICV)	Mid-point of the calibration curve with second source CRM	Recoveries: 70-130%	If the recovery for any analyte is outside of the acceptance criteria, recalibrate the instrument. Perform a root cause analysis.
Continued Calibration Verification (CCV)	One per every 10 samples. All analytes in each CCV must meet the criteria.	Recoveries: 80-120% (exempting analytes listed below) **70-130% for the following: -analytes with BP<0°C -methanol -acetonitrile -acetone -isopropyl alcohol -ethanol	Reanalyze all samples that preceded the last CCV that met the acceptance criteria. If CCV samples continue to fail, then recalibrate the instrument. ** These are common laboratory solvents found in cannabis laboratories for extractions for other analyses. The acceptance criteria are reflective of the potential low-level contamination.

Appendix F, Table 4: Batch QC Acceptance Criteria for Residual Solvents analysis

Calibration Criteria

- Basic calibration scheme
  - Gravimetric dilution – determine dilution factors for standards in g/g
  - Minimum number of calibration levels: 5 Levels
  - Calibration concentrations:
    - Laboratory shall determine appropriate concentrations for calibration standard levels given their relevant lists of analytes and the mixes available to them.
    - Laboratory must ensure that for the standard sample preparation, the concentration in solution that corresponds to the action limit for each analyte lies within the concentration range of the calibration curve. (e.g. for a 0.25 g sample, is the pass/fail concentration of an analyte within the range of your calibration curve?)
    - If possible, the concentration corresponding to the action limit should lie at a midpoint of the calibration curve.
  - Types of calibration and acceptance criteria:
    - Average Response Factor
    - Linear or quadratic regression
    - Weighted linear regression (up t1/x)
    - Calibration criteria: R2 ≥ 0.995 and RSE < 25%

25. Appendix G: Terpene Analysis

Terpenes are a class of compounds that are found occurring in nature, they are iterations on the same basic backbone, an isoprene unit $(C_5H_8)_n$. There are over 30,000 unique terpenes that have been found to exist. These special compounds lend plants their flavor or odor properties. Terpenes are further classified by the number of carbons: monoterpenes (C10),

sesquiterpenes (C15), diterpenes (C20), etc. Some common terpenes found in cannabis and other plants are limonene, the smell of citrus, pinene, the smell of pine needles, and beta-myrcene, present in mangoes.

Cannabis terpenes provide unique properties and potential interactions with cannabinoids that can influence the perception of effects. They also help biologists keep track of cultivars and phenotypes, using chemovar profiling by measuring the concentration of terpenes in a specific variety of cannabis. Knowledge surrounding terpenes is ever growing, and cannabis terpenes have brought some of these molecules and their properties into public discourse.

Analytes

Required Terpene Analytes[a] (chosen for their ubiquity in cannabis plant material and manufactured products)	
Compound	CAS Number
beta-Myrcene	123-35-3
beta-Caryophyllene	87-44-5
alpha-Pinene	80-56-8
beta-Pinene	18172-67-3
alpha-Humulene	6753-98-6
alpha-Bisabolol	515-69-5
Limonene	138-86-3
Linalool	78-70-6
Terpinolene	586-62-9
Caryophyllene oxide	1139-30-6
Additional Analytes	
	CAS Number
Delta-3-carene	13466-78-9
Camphene	79-92-5
p-cymene	99-87-6
Guaiol	489-86-1
Geraniol	106-24-1
Alpha-terpinene	99-86-5
Gamma-terpinene	99-85-4
Alpha & Beta Terpineol	98-55-5 (alpha), 138-87-4 (beta)
Cis- & Trans- nerolidol	3790-78-1 (cis), 40716-66-3 (trans)
Eucalyptol	470-82-6
Ocimene (variety of isomers, beta-Ocimene dominant)	3779-61-1 (E-beta), 13877-91-3 (Z-beta)
Borneol	464-43-7
alpha and beta Farnesene	502-61-4 (alpha), 18794-84-8 (beta)
Any additional terpenes, if labeled in a cannabis good	

[a]Adapted from Nevada cannabis regulation 11.055; most common terpenes found in cannabis

Appendix G, Table 1: Analyte list for terpene analysis
(list is not comprehensive, there are additional analytes found in cannabis not listed here)

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Concentration Considerations

- Range of concentrations that produce a linear calibration curve
- Routine Achievable LOQs: Generally ~ 10 ppm in sample
- Upper limit of the calibration curve should be defined by the concentrations of terpenes typically found in samples the laboratory is receiving.

Technology/Equipment/Supplies

- Instrumentation recommended: GC-MS, GC-MS-Headspace
- Instrument peripherals recommendations (Columns: 5-MS, 5-sil, rxi624 or equivalent)

Preparation

- Preparation Notes (direct inject, extraction headspace)
  - Sample Mass:
    - At least 0.5 grams
  - Solvents
    - Methanol
    - Ethanol
    - Isopropanol
  - Final extract
    - Final Volume: 10-30 mL

Batch QC Requirements

- Batch size: 20 samples maximum
- Batch QC/ Frequency/ Criteria
  - CCV - Prior to analysis, and one every ten samples thereafter
  - Reagent Blank - ⍰ LOQ - one per batch
  - Method Blank - one per batch
  - LCS - one per batch
  - Sample Duplicate - one duplicate sample per batch

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Batch QC Acceptance Criteria

Laboratory Control Sample	Frequency	Acceptance Criteria	Corrective Action
Method Blank/ Reagent Blank	Once each per batch	Non-detect for all analytes	Perform a root cause analysis to determine the source of contamination and remove it. Re-prep and reanalyze the entire analytical batch.
Laboratory Control Sample (LCS)	Once per batch.	Recovery 70% t130%	Re-prep LCS and reanalyze. If LCS fails again, determine if failure is a result of sample contamination or matrix effects, or due to improperly calibrated instruments. Remedy issue and reanalyze entire analytical batch.
Duplicate Sample	One per batch	RPD less than or equal t30 % between the duplicates.	Reanalyze sample and associated duplicate once. If the duplicates fail the precision criteria, determine if the error was instrumental or human. If human error, re-prep samples and reanalyze. If instrument error, remedy issue and re-analyze entire analytical batch.
Matrix Spike	Any new matrices not validated	Recovery 70% t130%	
Initial Calibration verification (ICV)	Directly after calibration.	Recovery 70% t130%	If the recovery for any analyte is outside of the acceptance criteria, recalibrate the instrument. Perform a root cause analysis.
Continued Calibration Verification (CCV)	Prior to analysis, and every 10 samples thereafter.	Recovery 70% t130%	Reanalyze all samples that preceded the last CCV that met the acceptance criteria. If CCV samples continue to fail, then recalibrate the instrument.

Appendix G, Table 2: Batch QC acceptance criteria for terpene analysis

Calibration Criteria

- Basic calibration scheme
  - Number of calibration levels: 5
  - Calibration concentrations
    - Range of concentrations that produce a linear calibration curve
    - Desired lowest LOQ ~ 10 ppm in sample
    - Upper limit of the calibration curve should be defined by the concentrations of terpenes typically found in samples the laboratory is receiving.
  - Type of calibration
    - Direct inject: Linear
    - Headspace: Linear or quadratic

- Calibration criteria: r^2 > 0.98 (linear), r^2 > 0.99 (quadratic). Perform a least squares error analysis to verify goodness of fit.
- Calibration frequency: analyze and trend & track CCV results to determine the validity of your calibration

- ICV: should be run immediately after calibration, and should be midpoint of the curve
- CCV: should be run prior to analysis, and one every ten samples thereafter

26. Appendix H: Water Activity Analysis

Water activity are two analytical procedures to measure the amount of water in a sample. In cannabis and hemp labs, the two procedures are performed for distinct and different reasons. Water activity is useful mainly in determining the ability of microorganisms to grow on the sample.

Technology/Equipment/Supplies

- Instrumentation recommended:
  - Water activity meter using a chilled mirror dew point analysis
  - Other water activity meter technologies

Generally aw< 0.65 is desired for inhalable products and < 0.85 for edible products.

Water Activity Methodologies Recommended:

- *USP <922> Water Activity*
- *ASTM D8196-20: Standard Practice for Determination of Water Activity in Cannabis Flower*
- *AOAC 978.18-1978 Water activity of canned vegetables*
- *USP <1112> Application of Water Activity Determination to Nonsterile Pharmaceutical Products*

Batch QC Requirements

- Max batch size 20 samples
- CCV or LCS (depending on the system suitability)
  - Frequency: at the start of batch, and every ten samples thereafter
- Duplicate samples
  - Frequency: once per batch
- Reference **Appendix H, Table 1** for Quality Control Sample Acceptance criteria requirements

Additional QC Recommendations

- Participate in a PT program at least annually.
- Measure and monitor environmental conditions such as the relative humidity and temperature. Establish acceptable criteria suitable for environmental conditions where you perform the water activity analysis, consider how these variables may be affecting your water activity measurements. If your environmental conditions are found to affect the measurements considerably then identify the contributing factors and adjust the environmental conditions until they return to acceptable ranges.

Batch QC Acceptance Criteria

Quality Control Sample	Water Analysis Method	Frequency	Acceptance Criteria	Corrective Action
Continued Calibration Verification (CCV)	Water Activity Standards	Two CCVs, bracketing the action limits	95-105 % Recovery	Perform a root cause analysis to attempt to identify the source of the failure. Remediate and run CCV again. If CCV fails a second time, service instrument.
Laboratory Control Sample (LCS)	Run a gauze sample that is saturated with one of the Water Activity Standards (comes as a kit)	One per batch at a midpoint concentration, unless a duplicate sample cannot be run, then add a second LCS.	95-105 % Recovery	Perform a root cause analysis to attempt to identify the source of the failure. Remediate and run the LCS again.
Duplicate Sample	Run one duplicate per batch.	One per batch	< 5 % Relative Percent Difference	Homogenize sample again and reanalyze.

Appendix H, Table 1: Batch QC Acceptance Criteria for water activity analysis

- Water activity meters typically come calibrated from the factory
    - The % recovery of a CCV sample can give you information about the continued validity of your calibration. If the instrument is found to be out of calibration, then either perform a calibration procedure as detailed in the manual or take the instrument out of service and send it to a manufacturer for calibration.

REVISION HISTORY

Revision Level	Description
Original Release	Original release.

ACKNOWLEDGMENTS

The Independent Laboratories Institute (ILI), would like to acknowledge the following ACIL/ILI member companies for their role as authors in this guide:
Anresco Laboratories, Agilent Technologies, Infinite Chemical Analysis Labs, Eurofins US, Restek Corporation, Kaycha Laboratories and Millipore Sigma..

The Independent Laboratories Institute (ILI) is also grateful to the following ACIL/ILI member companies for their role in reviewing and for their editorial contributions to this guide: AOAC, Foreign Trade Service Corporation, Susan Audino and Stawick Laboratory Management.

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