COLORADO
Department of Public Health & Environment

Dedicated to protecting and improving the health and environment of the people of Colorado

Reference Methods for the Testing of Retail and Medical Marijuana

Introduction

Cannabis is a novel industry and, currently, no recognized standard methods exist for the testing of cannabis or cannabis products. The purpose of this document is to provide guidance to testing facilities on the selection of applicable methodology pertaining to the testing of retail and medical marijuana/marijuana products. The methods outlined in this library are applicable by comparative use in areas similar to the testing of cannabis product; it should not, however, be construed that these methods are necessarily fit-for-purpose in all aspects of cannabis testing. The references outlined in this document lack complete validation or matrix extension specific to cannabis products and thus, require in house laboratory validation prior to implementation. Any selected methods must be shown as fit-for-purpose through in-house validation. The potency methods referenced for the analysis of marijuana/marijuana products are not derived from applicable standard methods as no proper standard method is available. Any method employed that was not derived from a standard method must be rigorously tested and validated prior to analysis of cannabis and cannabis product.

Note: The sources listed in this document are not exhaustive; other methodologies may be appropriate for use. Due to the constant evolution of scientific analytical methods, this reference library represents a living document that will be updated as needed. Marijuana testing facilities are encouraged to consult with the CDPHE certification program during selection and implementation of testing methodologies.

Microbial Pathogens and Total Yeast and Mold

> Concerning the testing of cannabis product for microbiological contaminants, there is a large pool of standard methods on which to draw. All microbiological methods employed must include applicable controls. Qualitative pathogen methods must confirm presumptive results as either positive or negative by the inclusion of a confirmation step. Confirmation of pathogens should not be addressed by simply re-running positive sample enrichments or retesting remaining sample.

Methods applicable to *Salmonella* spp. and Shiga toxin-producing *Escherichia coli* testing:

- Association of Analytical Communities (AOAC) 2016. "Salmonella in Foods, 967.25" http://www.eoma.aoac.org/methods/info.asp?ID=47595
- Food and Drug Administration (FDA), 2016. Bacteriological Analytical Manual (BAM). http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
- International Standards Organization (ISO), 2002. "ISO/TS 6579:2002." http://www.iso.org/iso/catalogue_detail.htm?csnumber=29315
- International Standards Organization (ISO), 2012. "ISO/TS 13136:2012." http://www.iso.org/iso/catalogue_detail.htm?csnumber=53328
- Salfinger, Yvonne and Tortorello, Mary Lou, 2015. *Compendium of Methods for the Microbiological Examination of Foods, 5th Edition*. American Public Health Association.
- United States Department of Agriculture: Food Safety and Inspection Service (USDA FSIS), 2016. Microbiology Laboratory Guidebook. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

Methods applicable to total yeast and mold testing:

- Association of Analytical Communities (AOAC) 2016. "Yeast and Mold Counts in Foods, 997.02." http://www.eoma.aoac.org/methods/info.asp?ID=46847
- Food and Drug Administration (FDA), 2016. Bacteriological Analytical Manual (BAM). http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
- Salfinger, Yvonne and Tortorello, Mary Lou, 2015. *Compendium of Methods for the Microbiological Examination of Foods, 5th Edition*. American Public Health Association.

Residual Solvent Testing

Concerning residual solvent testing, there is a large pool of standard methods from which to draw. All methods employed must include applicable controls.

Methods applicable to residual solvent testing:

- American Society for Testing Materials (ASTM), 2016. https://global.ihs.com/standards.cfm?publisher=ASTM&RID=Z56&MID=ASTM&gclid=CJTImMLNosoCFQIHaQodhgoHkQ
- Environmental Protection Agency (EPA), 2016. "310B-Residual Solvents." http://www3.epa.gov/ttn/emc/methods/method310b.html
- Lake, Rick., 2016. "RESTEK Revised USP 467 Residual Solvent Method." RESTEK: http://www.restek.com/Technical-Resources/Technical-Library/Pharmaceutical/pharm_A017

- United States Pharmacopeia (USP), 2008. "<467> Residual Solvents." http://www.usp.org/usp-nf/official-text/accelerated-revision-process/accelerated-revision-history/general-chapter-organic-volatile

Pesticide Residue Testing

Concerning pesticide testing, there is a large pool of standard methods from which to draw. All methods employed must include applicable controls.

Methods applicable to pesticide residue testing:

- Association of Analytical Communities (AOAC) 2016. "Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate, 2007.01" http://www.eoma.aoac.org/methods/info.asp?ID=48938
- Collaborative Validation of the QuEChERS Procedure for the Determination of Pesticide Residues in Food by LC-MS/MS. J.Agric.Food Chem, 2011,59,6383-6411.
- Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study LEHOTAY: Journal of AOAC International Vol.90,No.2,2007.
- Food and Drug Administration (FDA), 2016: Pesticide Analytical Manual (PAM). http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006955.htm
- International Standards Organization (ISO), 2016. http://www.iso.org/iso/home.html
- United States Department of Agriculture: Food Safety and Inspection Service (USDA FSIS), 2016. Chemistry Laboratory Guidebook. http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/chemistry-laboratory-guidebook

Potency Determination

Concerning potency analysis, while several published methods exist, the available methods have not been validated to the level of a standard method. The following is a list of appropriate reference methods. Potency methods must be validated extensively to ensure they meet the requirements of testing.

Methods applicable to potency determination:

- Backer, Benjamin De., et al., 2009. Innovative development and validation of an HPLC/DAD method for the qualitative and quantitative determination of major cannabinoids in cannabis plant material. *Journal of Chromatography B*, 887 4115-4124.
- Bovens, Michael., et al., 2009. Recommended method for the identification and analysis of cannabis and cannabis products: manual for use by National drug analysis laboratories. United Nations. https://www.unodc.org/documents/scientific/ST-NAR-40-Ebook.pdf

- Gambaro, Veniero., et al., 2002. Determination of primary active constituents in Cannabis preparations by high-resolution gas chromatography/flame ionoization detection and high-performance liquid chromatography/UV detection. *Analytica Chimica Acta* 468, 245-254.
- L. Ambach, F. Penitschka, A. Broillet, S. König, W. Weinmann., 2014. Simultaneous quantification of delta-9-THC, THC-acid A, CBN and CBD in seized drugs using HPLC-DAD. *Forensic Science International* 243, 107-111.
- Stolker, A.A.M., et al., 2004. Determination of cannabinoids in cannabis products using liquid chromatography –ion trap mass spectrometry. *Journal of Chromatography A*, 1058, 143-151.
- Swift, Wendy., et al., 2013. Analysis of Cannabis Seizures in NSW, Australia: Cannabis Potency and Cannabinoid Profile, *PLOS One* v.8 i.7 e70052.
- Upton, Roy., et al., 2014. Cannabis Inflorescence Cannabis Spp.: Standards of Identity, Analysis, And Quality Control. American Herbal Pharmacopoeia.

Validation Guidelines

- Any method derived from a standard method or literature method requires validation showing that the method is fit to purpose. In the absence of standard methods, a single laboratory validation or equivalent is required to show that the method is fit for purpose in the intended matrix and, if applicable, that any modifications to the original method do not negatively impact performance. Method validation should, at a minimum, verify accuracy, precision, analytical sensitivity, analytical specificity, limit of detection, limit of quantification, reportable range and the identification of interfering substances. For microbiological methods adopted from a standard method, inclusivity/exclusivity does not require complete reassessment, provided that the referenced media, primers, probes, antibodies, critical chemistries, etc., were not modified.

  - Association of Analytical Communities (AOAC) 2012. "Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces." AOAC: http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/AOAC_Validation_Guidelines_for_Food_Microbiology-Prepub_version.pdf
  - Association of Analytical Communities (AOAC) 2002. "Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals" AOAC: http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf
  - Food and Drug Administration (FDA) 2015. "Analytical Procedures and Methods Validation for Drugs and Biologics: Guidance for Industry." FDA: http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm386366.pdf
  - Food & Drug Administration Office of Foods and Veterinary Medicine (FDA) 2015. "Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds 2nd Edition." FDA:

http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM298730.pdf

- International Conference on Harmonization (ICH) 1996. "Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology." ICH: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
- United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) 2010. "Guidance for Test Kit Manufacturers, Laboratories: Evaluating the Performance of Pathogen Test Kit Methods." USDA: http://www.fsis.usda.gov/shared/PDF/Validation_Studies_Pathogen_Detection_Methods.pdf