CANNABIS POTENCY:

AN EXAMPLE OF APPLYING ISO 17025 PRINCIPLES TO A TEST METHOD

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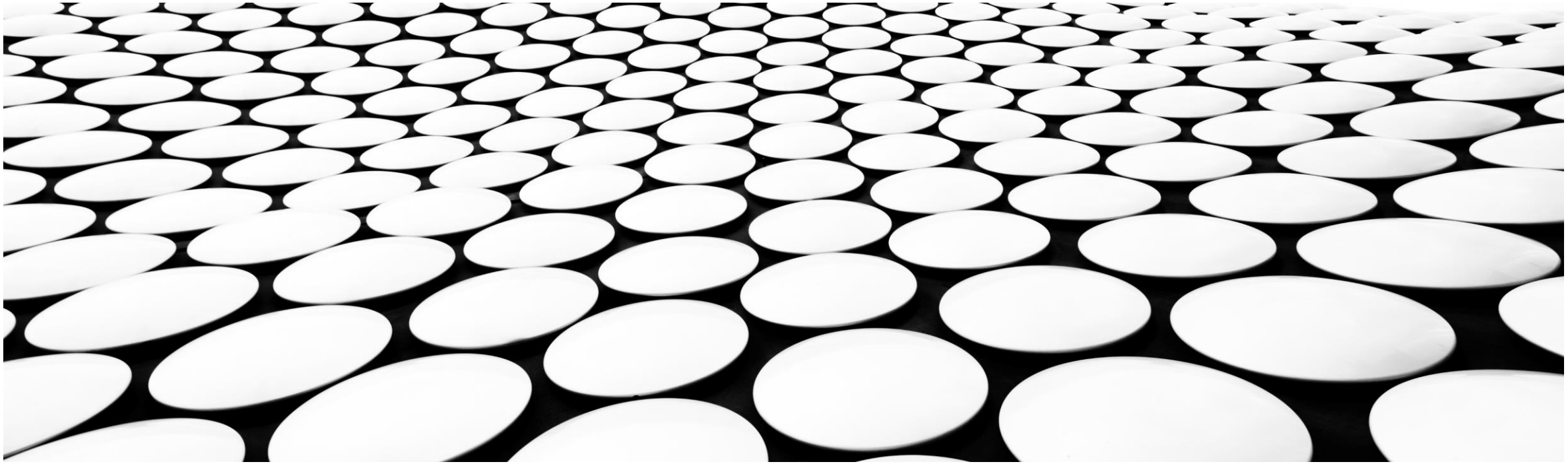
ON BEHALF OF PJLA, INC. - APRIL 13, 2020



VERIFICATION I

- "3.8 verification: provision of objective evidence that a given item fulfils specified requirements"
  - "Note 5 to entry: Verification should not be confused with calibration. Not every verification is a validation (3.9)."

- Possible verifications for cannabis potency:
  - High, medium, and low check standards for all analytes (pristine matrix, different from calibration check)
    - ISO 17034 > IUPAC (NIST) Traceable > ISO 17025 Producer with full Measurement Uncertainty > ISO 17025 with uncertainty statement > previous PT/ILS > Reputable distributor with uncertainty statement > "Wild West" >> Nothing
  - Example statement for SOP: "Once per batch, or every 20 samples for batches larger than 20, the analyst shall assess a standard material with known concentrations of reportable analytes (here CBDA, CBD, THCA, and THC) and assess results against the known values. When the analyzed quantities for these standards fall outside of ±5 % relative for the known values, the Laboratory Manager shall be consulted and informed. A value outside ±10 % relative to the known value shall constitute a batch failure, with troubleshooting (see section XXX) undertaken before reanalysis."

VERIFICATION II

- “3.8 verification: provision of objective evidence that a given item fulfils specified requirements”
  - “Note 5 to entry: Verification should not be confused with calibration. Not every verification is a validation (3.9).”

- Possible verifications for cannabis potency:
  - QCS/LCS - Quality Control Samples/Laboratory Control Samples (like-for-like matrix)
    - Matrix similar to the materials tested
  - Example statement for SOP: “Once per batch the analyst shall assess a previously analyzed material with known concentrations of reportable analytes (here CBDA, CBD, THCA, and THC) and assess results against the known values. When the analyzed quantities for these standards fall outside of ±10 % relative for the known values, the Laboratory Manager shall be consulted and informed. A value outside ±20 % relative to the known value shall constitute a batch failure, with troubleshooting (see section XXX) undertaken before reanalysis.”

VERIFICATION III

- “3.8 verification: provision of objective evidence that a given item fulfils specified requirements”
  - “Note 5 to entry: Verification should not be confused with calibration. Not every verification is a validation (3.9).”

- Possible verifications for cannabis potency:
  - Duplicate Analysis
    - Repeatability of live samples
  - Example statement for SOP: “Once per batch the analyst shall assess a first live sample twice (duplicate analysis) and assess results as relative percent differences (RPD). When the analyzed quantities fall outside ±5 % RPD, the Laboratory Manager shall be consulted and informed. A value outside ±10 % RPD shall constitute a batch failure, with troubleshooting (see section XXX) undertaken before reanalysis.”

VERIFICATION IV

- “3.8 verification: provision of objective evidence that a given item fulfils specified requirements”
  - “Note 5 to entry: Verification should not be confused with calibration. Not every verification is a validation (3.9).”

- Possible verifications for cannabis potency:
  - Calibration Checks
    - Reanalysis of an initial calibration standard
  - Example statement for SOP: “Prior to analysis of live samples an initial calibration verification (ICV) check shall be made using one of the calibration standards used in calibration. Every 10 samples and at the end of the batch (“bracketing”) a continuing calibration check (CCV) shall be made using the same sample used for the ICV. When the analyzed quantities for these checks fall outside of ±5 % relative for the known values, the Laboratory Manager shall be consulted and informed. A value outside ±10 % relative to the known value shall constitute a batch failure, with troubleshooting (see section XXX) undertaken before reanalysis.”

VERIFICATION V

- “3.8 verification: provision of objective evidence that a given item fulfils specified requirements”
  - “Note 5 to entry: Verification should not be confused with calibration. Not every verification is a validation (3.9).”

- Possible verifications for cannabis potency:
  - Laboratory Blanks
    - Analysis of a blank, analyte free sample advanced through the preparation process with the current batch
  - Example statement for SOP: “Prior to analysis of live samples a laboratory blank shall be made using a DI water blank that was prepared in the same fashion as the samples. If the results of this blank provide detectable quantities of any analyte, the Laboratory Manager shall be consulted and informed. A value above the reporting limit for this test shall constitute a batch failure, with troubleshooting (see section XXX) undertaken before reanalysis.”

VERIFICATION VI

- Example Batch of 40 for Cannabis Potency by HPLC-UV
  - ICAL -Calibration (injection 1-5)
  - ICV (injection 6)
  - LB (injection 7)
  - QCS (injection 8)
  - Sample 1 (injection 9)
  - Duplicate - Sample 1 (injection 10)
  - Sample 2-10 (injection 11-19)
  - CCV (injection 20)
  - Sample 11-20 (injection 21-30)
  - CCV (injection 31)
  - QCS (injection 32)
  - Sample 21-30 (injection 33-42)
  - CCV (injection 43)
  - Sample 31-40 (injection 44-53)
  - CCV (injection 54)

VERIFICATION VII

- "6.2.6 The laboratory shall authorize personnel to perform specific laboratory activities, including but not limited to, the following: (a) development, modification, verification and validation of methods"
- "6.4.13 Records shall be retained for equipment which can influence laboratory activities. The records shall include the following, where applicable: (c) evidence of verification that equipment conforms with specified requirements"
- "7.2.1.5 The laboratory shall verify that it can properly perform methods before introducing them by ensuring that it can achieve the required performance. Records of the verification shall be retained. If the method is revised by the issuing body, verification shall be repeated to the extent necessary."

- Example of "easy to objectively assess" practices
  - Ongoing, regularly assessed SPC/SQC program drawing on the QCS and ICV/CCV data.
  - Statement of verification and ongoing verification practices and resulting evidence.
  - Triggers for 'check with manager' and 'stop' and resulting evidence of practice.
  - Performing and meeting a well established initial and ongoing verification program (ASTM, AOAC, FDA/GLP, etc.).

CALIBRATION I

- “6.4.1 The laboratory shall have access to… measurement standards… required for the correct performance of laboratory activities and that can influence the results.”
- “NOTE 1 A multitude of names exist for reference materials and certified reference materials… Reference materials … are provided with a product information sheet/certificate that specifies, amongst other characteristics, homogeneity and stability for specified properties and, for certified reference materials, specified properties with certified values, their associated measurement uncertainty and metrological traceability.”
- The same level of preference exists for Calibration Standards as Verification Standards (slide 2).

CALIBRATION II

- Scope dictated:

  Have you called out a specific method, specific technology, general technology, or SOP?

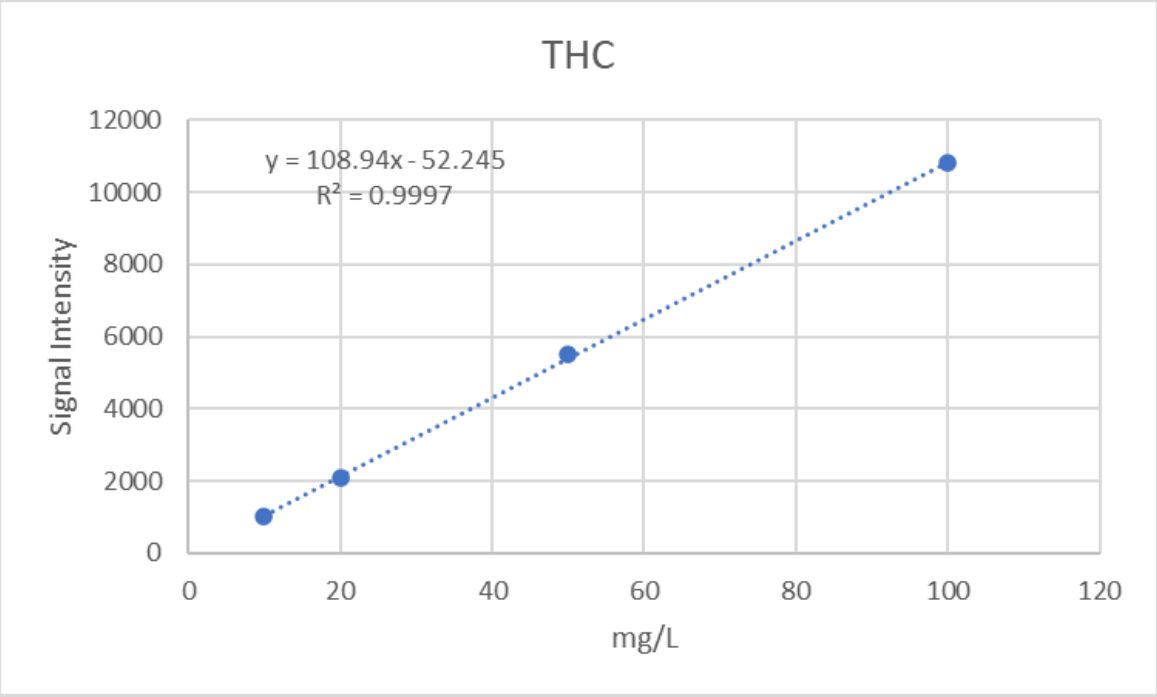
  - Best practices for a specific method can be sourced from the organization authoring the method (possibly in the method itself).
  - Good practices might include using the manufacturer's recommendation in the instance of a specific technology in the absence of other guidance.
  - Good practices might include the reference of a peer-reviewed study for general technologies in the absence of other guidance.
  - Good practices for an in-house method (SOP-based) might include the use of best practices from a respected body that is in alignment with state, local, or (eventually) federal guidance for methods based on the technology used.

CALIBRATION III

- Once you STATE a calibration regime, you are beholden to it.
- Sample Based:
  - Every batch, every 100 samples, every 200 injections…
- Time Based:
  - Every day, week, or month of operation.
- Event Based:
  - After batch failure, after PT failure, after PM…
- Regardless of regime - evidence should be available that the regime selected meets the needs of the process and the customer (e.g. Don’t just assume).

CALIBRATION IV

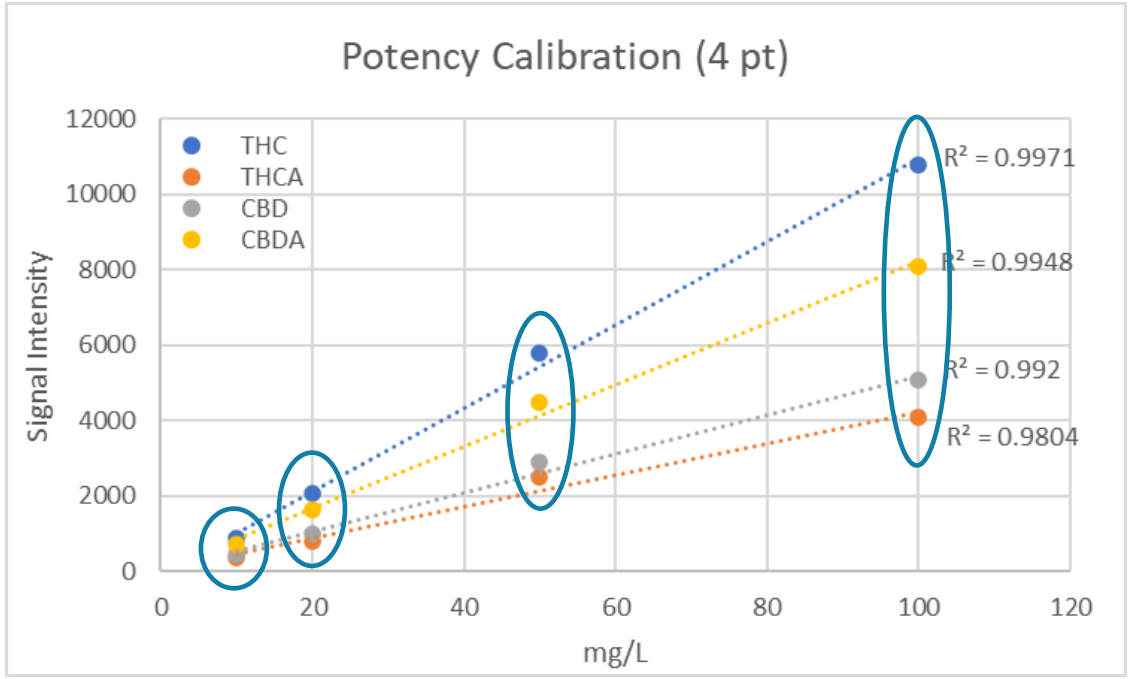
- Common issues in calibration
  - Recognition that deviation from the regression within the range/scope of the method can be the largest contributor to uncertainty.



THC		
mg/L	Signal	% Bias
10	1020	1.7%
20	2080	2.2%
50	5500	-1.9%
100	10800	0.4%

CALIBRATION IV

- Common issues in calibration
  - Obvious correlation in relative signals between analytes as you travel through the data set



When all the signals travel together in the calibration curve → Problem

Should each travel independently, with variance related to system noise

CALIBRATION IV

- Common issues in calibration
  - Insufficient range (must calibrate over reporting range/scope).
  - Consistent variance or bias of high, low, or middle standards.
    - Low concentration bias is often related to a need for background correction (biased high consistently).
    - High concentration bias is often related to detector saturation (biased low consistently).
    - Variability at a specific concentration may be associated with intermolecular interactions and critical points (consider co-solvent change).
  - Manual integration of any sort - indicates incorrect rates, ratios, column, or automation.
    - A good practice might be to treat a non-integrated peak during calibration as a PM trigger.
    - A good practice might be to treat all non-integrated analyte peaks as “Talk with Lab Manager” and/or “flag” events.
    - Will result in a finding for this method if automatic integration (the modern standard) is method and/or SOP mandated.

MEASUREMENT UNCERTAINTY I

- THIS IS THE FOUNDATION OF ALL CALIBRATION, VERIFICATION, AND RESPONSE TO OUTLIERS.
- “3.8 verification… EXAMPLE 3 Confirmation that a target measurement uncertainty can be met.”
- “6.4.1… Reference materials… are provided with a product information sheet/certificate that specifies, amongst other characteristics, homogeneity and stability for specified properties and, for certified reference materials, specified properties with certified values, their associated measurement uncertainty and metrological traceability.”
- **“6.4.5 The equipment used for measurement shall be capable of achieving the measurement accuracy and/or measurement uncertainty required to provide a valid result.”**
- “6.4.6 Measuring equipment shall be calibrated when… the measurement accuracy or measurement uncertainty affects the validity of the reported results.”

MEASUREMENT UNCERTAINTY II

- **"7.2.1.1 The laboratory shall use appropriate methods… for evaluation of the measurement uncertainty as well as statistical techniques for analysis of data."**
- "7.2.2.1… (f) evaluation of measurement uncertainty of the results based on an understanding of the theoretical principles of the method and practical experience of the performance of the sampling or test method."
- "7.2.2.3… Performance characteristics can include… measurement uncertainty of the results…"
- "7.5.1 The laboratory shall ensure that technical records for each laboratory activity contain… identification of factors affecting the measurement result and its associated measurement uncertainty…"

MEASUREMENT UNCERTAINTY III

- 7.6 Evaluation of measurement uncertainty
- 7.6.1 Objective evidence that you have identified MU contributors.
- 7.6.2 Objective evidence that you have performed evaluation of all MU for all calibrations.
- 7.6.3 Objective evidence that you have performed evaluation of all MU for all tests.

MEASUREMENT UNCERTAINTY IV

- Example of a living and robust MU Statement:

  “Accuracy Statement: For all reported values of this method, the uncertainty of the reported value shall be taken as ±1.96 standard deviations of the last 20 QCS results - expressed as a percentage (%) of the reported values. The bias for the method shall be taken as the difference between the average of the last 20 QCS and the known value - expressed as a percentage (%). The precision of the method shall be taken as ±1.96 standard deviations of the RPD (%) results for the most recent 20 sets of duplicates.”

- Example of a static MU Statement:

  “Accuracy Statement: The uncertainty of the reported values of this method are analyte dependent and based on a verification study (Appendix I) in which the four analytes were assessed in batches of 7 over 5 days by multiple analysts. The study presents method repeatability (precision) as the average of the in-batch variation plus 2 standard deviations of the 5 results for those batches (established for each analyte). The study presents method reproducibility (accuracy) as 2 standard deviations of all results (established for each analyte). The study presents method bias as the offset between the known and the average of all measurements (established for each analyte).”

MEASUREMENT UNCERTAINTY V

- Example of a method dependent MU Statement:

  “Accuracy Statement: As per Method WXYZ issued from Group ABC, the accuracy of the method is X. Laboratory performance is assessed annually as per SOP 123 to confirm that measurements are at least as accurate as the method accuracy statement.”

- Example of a professional body-based MU Statement:

  “Accuracy Statement: The laboratory uses Guide WXYZ issued from Group ABC to assess the accuracy for this method. The most recent accuracy studies are held by the Quality Manager.”

MEASUREMENT UNCERTAINTY VI

- Worst state to be in - “We didn’t do it because we didn’t understand it”.
- Very bad state - “Here are our PT results”.
- Bad state - “Here are the records for analysis at install”.

- Okay state - “Here are our summary statistics for QC samples for this method”.

- Good state - “Here are our QC statistics, SPC charts, and records of review”.
- Better state - “Here are our records for identifying potential contributors to MU on this method, QC statistics, SPC charts, records of review, and records of periodic MU studies,”
- Best state - “Here are our records for identifying potential contributors to MU on this method, QC statistics, SPC charts, records of review, records of periodic MU studies, documentation of the MU for our standards, and an RMS-based estimate of our measurement system’s MU.”

CAPSTONE:
IDENTIFICATION OF DEVIATIONS AND RESPONSE TO DEVIATIONS

- Call out what a deviation or outlier is.
- Call out who, what, when, and why - and record it every time.
  - Possible SOP section - “All deviations detailed in the calibration, quality control, and the batch structure sections of this SOP shall be brought to the attention of the Laboratory Manager or their delegate. All deviations and corrective actions shall be recorded and captured in the method journal (DOC 123). Additionally, if the specialized knowledge or experience of the analyst causes the analyst to question a result, that analyst should also discuss the finding with the Laboratory Manager.”
- Keep a detailed record of decision making.
- If you don’t have ongoing verification and knowledge of measurement uncertainty then you don’t have any basis for identification, response, and correction.
- If you don’t know your performance, you cannot improve it or know when it has slipped.

QUESTION AND ANSWER

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