WAC 16-309-260 Method validations. (1) Laboratories must perform method validation studies prior to implementing a new or original test method, implementing an approved method, implementing a new instrument, or modifying an existing method or instrument for each matrices tested.

(2) The records must include sufficient information to allow for a comprehensive review of the studies performed. Laboratories must have criteria for acceptance of study data, for agreement of replicate study samples, and for defining true outlier values. Study samples for quantitative methods must meet the same qualitative criteria (e.g., the same retention time, mass ratio, internal standard abundance, and chromatography criteria) used for samples. The laboratory's acceptance criteria must be described in the SOP and in the study summary.

(3) Laboratories must perform reverification studies on an annual basis at minimum on high complexity nonreagent methods. Reverification studies are designed to verify that the existing LOD, LOQ, and ULOL values are still valid and do not require laboratories to analyze the same number of samples that are required for full validation studies.

(4) If the laboratory modifies an existing test method or instrument parameter that affects the performance of the method, the revised method must be re-validated prior to use. If the modification is relatively minor, the validation studies may be focused on those parameters that have been affected.

(5) Validations must include linearity, precision, accuracy, LOD, LOQ, ULOL, carryover, selectivity/interference, and matrix effects. unless defined specifically below.

(6) The laboratory must characterize the linearity of a method based on replicate analysis (i.e., a minimum of three replicates at each concentration) of samples of at least six concentrations. The concentrations must be distributed above and below the cutoff for the test.

(7) The laboratory must characterize the precision of a method based on replicate analysis, at least 20 results total. Analysis must be at significant concentrations around the cutoff/decision point and expected range. At least three replicates at each concentration must be analyzed. Precision studies must be performed on multiple days and in multiple batches in order to assess intra-batch and inter-batch variability.

(8) The laboratory must characterize the accuracy (expressed as bias) of a method by calculating the percent difference between the analyzed sample results and the target concentrations. Accuracy studies must be performed on multiple days and in multiple batches to assess intra-batch and inter-batch variability.

(9) The laboratory must characterize the LOD of a method by a series of replicates with decreasing concentrations (i.e., a minimum of three replicates at each concentration). The LOD must be experimentally determined and supported by analytical data. The laboratory can choose to artificially set the LOD at the established LOQ if the LOQ is at least 25 percent below the decision point limit.

(10) The laboratory must characterize the LOQ of a method by a series of replicates with decreasing concentrations (i.e., a minimum of three replicates at each concentration). The LOQ of a method must be determined and supported by analytical data and must be at least 25 percent below the decision point limit.

(11) The laboratory must characterize the ULOL of a method by a series of replicates with increasing concentrations (i.e., a minimum of three replicates at each concentration). Laboratories may select a

value at the upper end of the dynamic range for a method, but it must be determined and supported by analytical data.

(12) The laboratory must investigate the potential of carryover of a method from one sample to another during testing by analyzing highly concentrated samples followed by negative samples (i.e., without the analyte of interest) and evaluate the negative samples for carryover. Positive samples that follow a sample at carryover concentrations must be reinjected or reextracted to eliminate carryover concerns.

(13) The laboratory must investigate the day-to-day precision using positive and negative samples assuring the ruggedness of the testing method provides good reproducibility over a period of at least five days.

(14) The laboratory must investigate the selectivity and interferences of a method by testing commonly encountered compounds and compounds that are structurally similar that could potentially interfere with the method at higher concentrations. Laboratories may accept manufacturer studies of immunoassay products if the study was performed using cannabis-focused compounds.

(15) The laboratory must investigate any possible matrix effect by evaluating the potential for components of the sample matrix to either suppress or enhance the ionization of the analytes of the compound(s) of interest and internal standard(s). Studies must include the evaluation of at least five different lots of products (i.e., flower from five different plants or from five different plant lots).

(16) When dilution of a sample is necessary to keep the result concentration within the range of linearity, the laboratory must conduct dilution integrity studies to document that the dilution does not affect the method's performance. These consist of precision/accuracy studies using samples at the dilution specified in the procedure.

(17) The laboratory must perform a parallel study when a new instrument or a new/revised procedure is implemented where results from the revised/new method or new instrument are compared to results from the existing method/instrument.

(18) The laboratory must perform a positive/negative differentiation study when validating a qualitative test by analyzing positive and negative samples that have been verified by a quantitative method to assess the assay's ability to differentiate positive and negative samples. The laboratory may analyze a combination of positive and negative controls, proficiency test (PT) samples or previously tested samples. The laboratory must analyze a minimum of five positive samples at differing concentrations and five negative samples (i.e., 10 results total).

(19) The laboratory must verify extraction efficiency assuring their method can sufficiently extract out the analyte of interest from the sample matrix.

(20) Records for validation and periodic reverification studies must be organized in a format to facilitate a comprehensive review and, at a minimum, the records must include:

(a) A stated purpose;

(b) Description of test method(s);

(c) Identity of the instrument(s) used for the study;

(d) A listing of the instrument parameters used for the study;

(e) A description of the study samples;

(f) A summary of the statistical data collected to characterize the assay;

(g) A discussion;

(h) A summary with conclusions; and

(i) All raw analytical data from the samples analyzed in the study.

(21) The laboratory must use the same criteria for acceptance of study data (e.g., the same retention time, mass ratio, internal standard abundance, and chromatography criteria) as used for the daily samples.

(22) The laboratory must maintain the original assay validation study records for methods in production for an indefinite period. Validation and reverification study records must be made available at the time of inspection or upon request. Labs are required to maintain records for retired methods for five years.

(23) All immunoassay and qualitative assay methods must be properly validated prior to use with samples and supported with the following studies:

(a) Linearity;

(b) Precision and accuracy around the cutoff;

(c) Selectivity;

(d) Carryover;

(e) A parallel study using the existing and new/revised procedures;

(f) Positive/negative sample differentiation studies.

(24) All quantitative assays must be properly validated prior to use with samples and supported with the following studies:

(a) Determination of LOQ, LOD, and ULOL;

(b) Precision/accuracy around the cutoff;

(c) Carryover;

(d) Selectivity/interference;

(e) For an assay validation: Method parameters including ion selection;

(f) For full instrument validation: Instrument parameter optimization;

(g) For LC-MS, and LC-MS/MS methods: Matrix effects;

(h) For assays using a new technology: Parallel studies of PT samples and customer samples (e.g., when validating a technology different from the existing method);

(i) For assays using an extraction: Extraction efficiency must be determined; and

(j) Hydrolysis efficiency (if sample preparation includes a hydrolysis step).

(25) An abbreviated instrument validation must be performed prior to implementing an additional instrument of an exact model that has been validated by the laboratory. The laboratory must perform the following studies:

(a) Determination of the LOQ, LOD, and ULOL;

(b) Carryover evaluation;

(c) Instrument parameter optimization; and

(d) For LC, LC-MS, and LC-MS/MS methods: Evaluation of matrix effects.

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