

Cover Sheet (Page 1)

Single Laboratory Validation (Chemistry)

-- Generic Protocol --

1. Study Title: *Single Laboratory validation of the determination of [analyte] in [matrix] by [nature of determination]*
2. SLV Coordinator/Performing Laboratory (*address, telephone and FAX numbers, e-mail*)
3. Laboratory Project Identification
4. Date

Study Identification (Page 2)

Study Identification

1. Study Title:
2. Study Objective: *The objective of this study is to validate an assay for ----- in -----.*
3. SLV Coordinator/ Performing Laboratory
4. Study Monitors (*AOAC General Referee, Expert Review Panel Chairman, Senior Scientific Administrator*)
5. Test Materials (*List provided by AOAC*)
6. Method Reference (*if published, if not supply a copy of the method to be validated*)
7. Proposed Timetable
 - a. Initiation date
 - b. Completion of study protocol
 - c. Review/approval of study protocol
 - d. Experimental start date
 - e. Experimental termination date
 - f. Draft report due date
 - g. Review of draft report
 - h. Preparation of final report based upon comments
 - i. Final report/manuscript submission for publication

Study Description (Page 3 and following)

1. **Scope:** This method is applicable to the determination (and/or identification) of _____ (analyte(s) in _____ (matrices) at concentrations of (x to y units) in the presence of _____ and in the absence of _____.
2. **Test Materials:** The test materials for this study will be _____. Negative controls to be used are _____. *(Specify the source, homogeneity and stability of the test materials. Conduct a number of typical matrices with presumably “none” of the analyte present through the method to establish the expected baseline of reported analyte with “none” present.)*

In the case of plant materials, describe how the identity of the plant material(s) is established or authenticated, as well as how blank materials were obtained (exhaustively extracted test samples may be suitable), or related plant species not containing the analyte may be used if their composition is similar. Negative controls are essential if the limits of detection (LOD) and of quantitation (LOQ) must be established, since the standard deviation of the blank material will be used to calculate the limit. A Reagent blank is not suitable for this purpose.

In all cases, a reserve sample of the original parent material(s) should be stored for future reference.

3. **Reference Standards:** The following reference standards will be obtained from commercial sources: _____ *(Specify source, purity, stability and storage conditions. Availability of certified reference materials permits evaluation of laboratory and method bias together. Certification may be performed by an institution, supplier or user by direct assay, multiple laboratory analysis, or by impurity analysis by chromatography or spectrophotometry.*

If reference standards are not available, an internal standard may be used. In all cases, specimens of the materials should be preserved for comparison to later specimens if necessary.

4. Single Lab Validation Study Design:

4.1 Method: Give directions for performance of all analytical operations.

- 4.1.1 *Preparation of the test sample from the laboratory sample.*
 - 4.1.1.1 *The reduction of the laboratory sample to the test sample in amount (bulk) by quartering, riffing and sampling; and in fineness (by crushing or grinding).*
 - 4.1.1.2 *The dissolution of the test (analytical) sample in a solvent to provide a test solution.*

- 4.1.2 *Describe any modifications/optimizations made to the original method. Since ruggedness testing is usually undertaken during method development, describe any additional testing done in the application of the method to the matrices selected. The method should be optimized with respect to extraction efficiency (vary solvent, time and temperature) and sample/standard stability. Procedures for determining extraction efficiency are contained in reference 2*
- 4.1.3 *Description of instrumental parameters, calibration procedures, systems suitability tests*
- 4.1.4 *Description of selectivity and specificity studies.*

5. Single Lab Validation Parameters

5.1 **Calibration Curve:** Describe the relationship between the concentration of the isolated analyte and the signal from the measuring instrument. *Do not use the same reference material for the determination of bias and for calibration or the error estimate is cancelled out. The concentration ranges must cover the expected concentrations of the analyte in the samples to be analyzed. As a rule of thumb the calibration range will cover approximately 0.75-150% of the expected concentrations of each analyte in each test matrix. In the ephedra study at least 3 independent calibration curves were generated for each calibration range on separate occasions during the course of the validation study, and a calibration standard curve (minimum of 6 concentration levels) was run at a minimum before and after each set of samples.*

Note: Six levels before and after a set of analyses may imply a relatively unstable system. This seems excessive, although an automated system could easily handle it. The important thing is the requirement for a stable relationship that is reproducible and covers the range of interest. It need not be linear and it should not be characterized by a "high" correlation coefficient.

- 5.1.1 *Linearity: The calibration curve need not be linear. If the calibration curve is not linear, at least 3 concentrations are required (upper, lower and mid range).. For assistance in calculating correlation coefficients see reference 3. In the case of the ephedra study, the relative response of the analyte vs. the concentration were used to construct the calibration curves using the least squares linear regression method.*
- 5.1.2 *Calibration curve precision: The back-calculated values for each concentration should be within the targets expected depending on concentration (see Table below).*

5.2. Test Material Precision (Repeatability Standard Deviation): Describe experiments to be done. *A typical repeatability study design (reference 3) includes the performance of r replicate analyses of m test portions over a period of d days for each sample type (matrix), where r is the number of replicates (2,3,---); m is the number of test portions in each group, and n is the number of different sample types. As a general rule, $r \times m$ should never be less than 10; n should be at least 2, preferably more; and d should be at least 2. If a blank determination is needed (and is used in calculations), n replicates of the blank should be made in each group (day). Note that the purpose of the validation exercise is to obtain an estimate of representative variation to be expected in actual practice, not to demonstrate the “best” performance from the laboratory. It is better to obtain the variability “between days” by conducting 2 determinations on each of 5 days than 5 determinations on each of 2 days. Repeat the analysis of the same representative materials at least on 2 additional days or by at least 2 additional analysts to provide an estimate of the expected performance parameters from that laboratory. Typical expected precision limits are contained in the table below.*

5.3. Negative Control Recovery: Fortify samples of the negative control with the reference compound mixture, at 15%, 30% and 130% of the expected concentrations. *Analyze triplicate unfortified controls concurrently. Repeat on at least two additional occasions (cf. ephedra study).*

5.4. Accuracy: Describe what will be done. *The steps frequently employed in determination of accuracy (recovery) are: (a) preparation of a reference standard solution; (b) addition of known amounts of the reference standard solution to a weighed amount of the matrix blank. Multiple preparations at each level are required in order to determine the mean and standard deviation at each level (to isolate the random error from bias). At least 7 replicates are required for a reasonable estimate of the population mean. Frequently triplicate preparations at each level are prepared, and the experiment is repeated on 3 separate days (total of 9 replicates at each level). Typical acceptable recovery levels are found in the following table.*

Recommended recovery and precision limits for single lab validation:

Concentration	Repeatability (%)	Recovery (%)
100%	1	98-101
10%	1.5	95-102
1%	2	92-105
0.1%	3	90-108
0.01%	4	85-110
10µg/g (10 ppm)	6	80-115
1 µg/g (1 ppm)	8	75-120
10 ng/g(10 ppb)	15	70-125

5.5 Secondary and Relative Response Factors: *If needed, describe what will be done.*

5.6 Internal Standards: *If needed, describe what will be done.*

6. **Protocol Amendments, Protocol Deviations, and SOP deviations:** *All proposed protocol amendments, protocol deviations, and SOP deviations are to be documented by the test personnel and reported to the SLV Coordinator. All protocol amendments and deviations are to be documented in writing by the SLV Coordinator and submitted to the Sponsor for approval. All protocol amendments and deviations will be included in the final report.*
7. **Statistical Evaluation:** *Statistical analyses, where applicable, will ordinarily mean, standard deviation and relative standard deviation (RSD).*
8. **Report:** *A draft report will be submitted to the Sponsor for review and comment. Data generated from the study will be used to generate the final determinative and confirmation method to be submitted for full collaborative study. This report will include, but not be limited to the items following items: (a) analytical methods used in generation of the data; (b) all chromatograms from acceptable runs to include controls, standards, and samples; (c) instruments used and operating conditions; (d) protocol, amendments and deviations (as necessary); (e) test article information; (f) names of all scientists, professionals, and supervisory personnel involved in the study; and (g) summary tabular data and analytical spread-sheets for all acceptable runs. A final report will be provided the Sponsor in the form of a publication to be submitted to the J.AOAC Int.*

9. **Maintenance of Data Records and Specimens:** *Data to be transferred to and maintained will include, but not be limited to, the following items: (a) protocol; (b) amendments, if pertinent; (c) test article information; (d) test article analysis records; (e) laboratory notebook(s) containing results; (f) study correspondence; (g) sample analysis data; and (h) final report.*

All raw data, documentation, records, the protocol, amendments, reserve samples, and final report generated as a result of the study will be archived ----. Disposal of these articles will be at the discretion of the Sponsor.

10. **References:**

1. M.Thompson, S. Ellison and R. Wood, "Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis," Pure Appl. Chem. (5), 835-855 (2002).
2. AOAC, "Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements," April 2003.
3. AOAC INTERNATIONAL Training Course, "Single Laboratory Validation of Analytical Methods for Dietary Supplements, April 21-23, 2003.

11. **Signatures:**