

# COMPARISON of QUANTITATIVE CHEMICAL SINGLE LABORATORY VALIDATION (SLV) REQUIREMENTS

By Jo Marie Cook, Florida Department of Agriculture and Consumer Services

August 2009

## Overview:

I reviewed several different validation guidelines and summarized the requirements of each for validation of quantitative, multiresidue type chemical methods. I noted the minimum requirements for each. Even more replicates are necessary for validation of qualitative methods. The "Guidelines for SLV of Analytical Methods for Trace-Level Concentrations of Organic Chemicals", was the most complete and relevant guide. It was developed through a harmonization project between CODEX, ISO, AOAC, FAO, IAEA and IUPAC in 1999. I believe it remains the most important guide for trace level, multi-residue work. All the other guides are more general in nature. This guide also discusses extension of a method or performance verification by another laboratory. The only significant difference between this guide and others is that most guides recommend calibration for the range of concentrations expected. The CODEX and EU guides concentrated on ranges within 2 times the regulatory limit.

Most guides mention the need for SLV's and the decline of collaborative studies due to time and cost.

Despite frequent reference to "Fit for Purpose", the need for SLV's and the number of replicates is not significantly diminished for multiresidue methods. If anything, more detailed SLV's are important due to the scarcity of collaboratively studied methods. Some performance parameters needed to be measured only once such as selectivity, ruggedness and stability. Verification of method performance in other laboratories required validation parameters of accuracy and precision, similar to those in collaborative studies. However, most of the guides stressed the need to measure realistic repeatability (multiple days and sample types) and reproducibility (multiple analysts, instruments, reagents, etc). None of the guides mentioned interlab (3 - 5 lab) validations, presumably because this additional validation information is not statistically significant until 7 or more laboratories are involved.

For most guides, the number of replicates needed for accuracy and repeatability was three blank matrices plus three fortified levels, 5-6 replicates, repeated on three different days. The AOAC generic protocol was less stringent than any of the other guides.

Because certified reference materials are seldom available, trueness or accuracy must be determined by spiking multiple varieties of blank samples. Inefficiency of extraction can be the major source of bias. This makes it more important to evaluate method response with standard additions, comparison to alternate methods, proficiencies, evaluate ruggedness, stability etc. It is important to determine if the method will extract the analytes of interest in incurred samples in the same manner as demonstrated by fortified blank matrix recovery studies.

Most guides mentioned using Youden pairs to determine ruggedness. Variables which contribute significant imprecision should be corrected by additional method development.

## References:

### AOAC:

*AOAC Guidelines for Single Laboratory Validation of Chemical Method for Dietary Supplements and Botanicals*, <http://www.aoac.org/dietsupp6/Dietary-Supplement-web-site/DSHomePage2.html>, 2002-12-19

*Single Laboratory Validation, Generic Protocol (Chemistry)* - Draft 8, AOAC, A. Pohland, APohland@AOAC.ORG

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*Single Laboratory Validation Acceptance Criteria (Chemistry Methods) - Draft 8, AOAC, A. Pohland, APohland@AOAC.ORG*

*Protocols for AOAC Method Validation Programs - Rev 2, 3-17-2008, AOAC-OMB, G. Latimer, latimerstx@verizon.net*

## FDA-CVM:

*FDA Guideline 3 V: Guideline for Approval of a Method of Analysis for Residues, FDA, <http://www.fda.gov/cvm/Guidance/1731.htm>*

*FDA Guideline 118, Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues, <http://www.fda.gov/cvm/Guidance/guide118.pdf>*

*Second Analyst Validation of Study 419.01 - Multiclass Determination and Confirmation of Antibiotic Residues in Honey using LC-MS\_MS, Mayda Lopez, FDA CVM*

## EU:

*Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, Official Journal of the European Communities,*

HARMONIZED SLV: (AOAC, Food and Agriculture Organization of the United Nations, International Atomic Energy Agency, International Union of Pure and Applied Chemistry)

*Guidelines for Single-Laboratory Validation of Analytical Methods for Trace-Level concentrations of Organic Chemicals, AOAC/FAO/IAEA/IUPAC, 1999*  
[http://www.iaea.org/trc/pest-qa\\_val2.htm](http://www.iaea.org/trc/pest-qa_val2.htm)

ISO / IEC: (International Organization for Standardization & International Electrotechnical Commission)

*Guidelines for checking and validating test and calibration methods according to ISO/IEC 17025, A011, 1-9-2008, Ver. 4*

IUPAC (International Union of Pure and Applied Chemistry):

*Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis, IUPAC Report, Thompson, Ellison, Wood, 11-4-1999*

## EURACHEM:

*The Fitness for Purpose Analytical Methods, A Laboratory Guide to Method Validation and Related Topics, EURACHEM Working Group, David Holcombe, 1998, ISBN: 0-948926-12-0*

## GENERAL READING:

*Principles and Practices of Method Validation, edited by A Fajgelj and A. Ambrus, ISBN 0-85404-783-2, Royal Society of Chemistry 2000, [www.rsc.org](http://www.rsc.org)*

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Performance Parameters	AOAC <sup>8</sup>	FDA-CVM Guides 3.4, 145-chemistry, 118	EU <sup>9</sup>	Harmonized Multiresidue SLV Guide	ISO/IEC 17025 A011	IUPAC	EUROCHEM Guide
<b>DEVELOPMENT and OPTIMIZATION - to be conducted for new methods and may/or may not be included in SLV's by additional laboratories</b>							
Purpose	Fit for Purpose	Fit for Purpose				Fit for Purpose	Fit for Purpose
Test "System"						Method, conc. Range, matrix, use	
Selectivity or Specificity	3-4 MS fragments	Retention time <sup>7</sup> GC/MS=2%, LC/MS=5% 3-4 MS fragments Full scan 20% match SIM = 10% match MS x MS = 20% match	IP Points	5	required	Selectivity Index, matrix interference	Slope of calibration curve Spike interferences Other methods
Range of Blank matrices	2		20	5 representative commodities		Bias due to matrix suspected to be significant	
Range of related compounds (metabolites, derivatives,,)			Y	5			Zero + 6 levels Range of sample levels Linear level of instrument and method
Blank spiked w/ interference			Y				
False Positive	< 5%	zero	Y				
False Negative		< 10%	Y				
Quant. Enhancement or suppression			Y				
Calibration Curves	At least 3 levels		5 levels including zero	Zero + 3 levels times 2 - 10 LCL, AL, AL*2 Non-linear = 7 levels times 3 Matrix vs standards in solvent		6 levels 0 - 150%, evenly distributed Run in duplicate or triplicate in random order Test matrix effect w/ std. add. Plot residuals	Zero + 6 levels
Decision Limit (LD or LOD) Sensitivity	3 times SD or average of blank	3 times signal to noise	Calibration curve method 5 20 blanks, 3 times signal to noise	16 Zero, LCL, AL times 5 2-3 * AL times 3	Required	Estimate, only a rough guide 3 times SD of matrix blank	10 blanks or low level spikes Terms not generally accepted. "minimum detectable value" Blank + 3SD of lowest concentration
Detection Capability (LOQ)	10 time SD or average of blank		Decision limit plus 1.64 times the SD of the reproducibility	16 Zero, LCL, AL times 5 2-3 * AL times 3	Required	10% RSD ...or 2 times LOD Not recommended. State the uncertainty at concentration	10 blanks or low level spikes Blanks + 10 SD May not be lower than the lowest quantitative measure.
Stability (light, temperature...) Analytes in standard solution Incurred residue ...or matrix fortified			1, 2, 3, 4 weeks or more Store at -20C or less 0, 1, 2, 3, 4 weeks (-20C)	>5 each Analyte/commodity & standard solutions			
Optimization (re-extraction, sample preparation, alternate solvents, shake time/type, pH, detectors, cleanup, instrument, columns...)							
Ruggedness (minor changes)	Youden pairs	Youden pairs	Youden pairs				Youden pairs

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Comparison to established method							
<b>VALIDATION - basic measurements common to all levels of validation. Some SLV's may require some or all of the tests above.</b>							
Representative Sample Types	2 - 3 or more as needed to represent the range of composition	5	During development - 20				
Trueness (accuracy or bias) Certified Ref. Material ...or see Recovery			CVM		Required		10
Calibration Curves	3 levels (upper, lower, middle) (Dietary Supl = 0, 5-7 points)		5 levels including zero		Required Determine range		
Recovery <i>Analyst performing validation should not be aware of concentration / composition</i>	2 9 - 10 range of expected concentrations 0 * 3, 15%, 30%, 130% times 3 times 3 days	20 0.5, 1, 2 LOQ times 5 5 incurred	18 1, 1.5, 2.0 LOD times 6 3,4 ...or 0.5, 1, 1.5 Permitted Level times 6	Zero, LCL, AL times 5 2-3 * AL times 3  Representative analytes and matrices	Required	? 2 levels, min and max	18 Blanks + 3 levels times 6
Incurred residues	If possible						
Repeatability (% CV)	At least 10 At least 2 different sample types (Dietary Supl = 3 matrices) Times 2, At least 2 days	<20% Perform the required recovery experiments over 3 days Blinded samples	18 Recovery on 3 different days	Zero, LCL, AL times 5 2-3 * AL times 3  Representative analytes and matrices	Required	Duplicates times several days, range of concentration, different matrix Horwitz is incorrect < 120 ppb	10
Within-Lab Reproducibility or Intermediate Precision (%CV) (different reagents, analysts, rooms, instruments, days)	15- 20 Each condition, concentrations that differ by order of magnitude times 5		18 Recovery on 3 different days		Required		10 Different analysts, equipment, days
Measurement Uncertainty 6							Includes bias if uncorrected
Ruggedness (major changes)			Inter lab study				

Notes:

1. Experiments may be combined to determine different parameters
2. Multiple analytes can be determined at the same time as long as they do not interfere
3. LOD = minimum required quantitative performance limit of the method
4. Recovery is measured by method of standard additions (100 (conc. 2 -conc 1)/concentration added to #2)
5. Concentration at y intercept plus 2.33 times the SD or the reproducibility of the intercept  
...or 3 times signal to noise
6. Bias, as measured by recovery, is not a component of uncertainty. Bias (a constant) should be removed by subtraction before calculating MU standard deviations (AOAC Dietary Supplement Guidelines for SLV)
7. See FDA Guidance for Industry #118
8. AOAC general guidelines required fewer replicates and levels as recommended by the dietary supplement guide, which was more consistent with other guides.
9. The range of concentrations recommended for recovery and repeatability was narrow. Other guidelines recommended measurements across broader ranges.
10. per the CODEX guidelines: AL = Action Level or Acceptable Level (regulatory limit) and LCL = lowest calibration level