STREAMLINING ANALYTICAL METHOD VALIDATION ACROSS THE ORGANIZATION

SmartSTATS\Enoval integration into method development and validation workflows allows our customers to significantly improve the efficiency and quality of the validation processes.

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PHARMALEX
CONFIDENCE BEYOND COMPLIANCE

1. Define the Analytical Target Profile (ATP)

Starting point of validation is a clear ATP definition in function of the Total Analytical Error (TAE) as advised by USP 1220 and ICH Q14. For example:

The procedure must be able to quantify potassium bicarbonate in a range from 800 mg to 1200 mg in our pharmaceutical product so that 95% of all our future measured values fall within ±1.5% relative error range.

2. Determine the optimal validation design

Every validation attempt, due to inherent measurement error, has a probability to fail, even if the analytical method is truly fit for purpose. One can use a-priori knowledge about the analytical method's performance to estimate the probability of validation success for various designs as shown in the following table.

σw	0.1			0.2			0.3			0.4			0.5		
σb	n	p	P(success)	n	p	P(success)	n	p	P(success)	n	p	P(success)	n	p	P(success)
0.1	3	2	0.9980	3	2	0.9715	3	3	0.9606	3	5	0.9592	5	5	0.9612
							3	4	0.9847	3	6	0.9778	5	6	0.9808
							4	2	0.9759	4	4	0.9848	6	4	0.9605
										5	3	0.9869	6	5	0.9845
										6	2	0.9734			
0.2	4	2	0.9863	4	2	0.9617	4	4	0.9669	5	4	0.9583			
	4	3	0.9886	4	3	0.9793	4	5	0.9767	5	5	0.9748			
				4	4	0.9850	4	6	0.9820	5	6	0.9819			
				4	5	0.9882	5	2	0.9607	6	3	0.9621			
				4	6	0.9897	5	3	0.9874	6	4	0.9830			
							6	2	0.9873						
0.3	6	2	0.9761	6	2	0.9521	6	5	0.9528						
	6	3	0.9787	6	3	0.9655	6	6	0.9598						
	6	4	0.9802	6	4	0.9695									
	6	5	0.9813	6	5	0.9736									
	6	6	0.9813	6	6	0.9765									

Table 1: Recommended number of series (n), replicates by series (p) and the probability of a successful validation attempt, P(success), as a function of the expected values for the between-series (σb) and the within-series (σw) standard deviations (in %) when acceptance limits are set to 1.5 % and bias is set 0 %.

3. Compute calibration curves

Simple methods do not require calibration. But more complex methods such as ELISA need to be calibrated. Enoval offers 16 different calibration models to choose from.

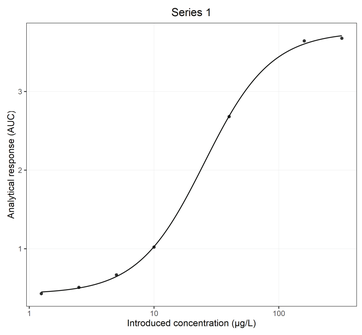

Figure 1: Four parameter logistic calibration curve on a logarithmic scale.

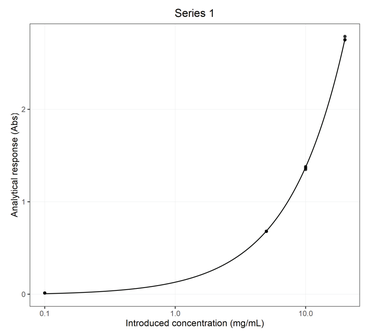

Figure 2: Power calibration curve on a logarithmic scale.

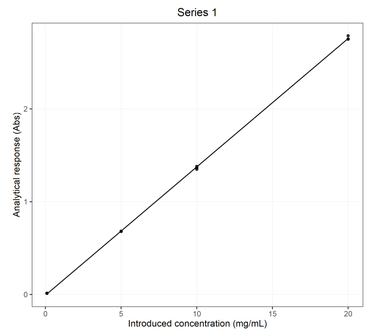

Figure 3: Simple linear calibration curve.

4. Compute trueness and precision

Balanced design or not: no need to bother with formulas. Our software will always use the correct statistical approach to provide you with correct statistical results.

Amount level (%)	Mean introduced amount (mg)	Mean result (mg)	Absolute bias (mg)	Relative bias (%)	Recovery (%)	95% Confidence Interval of Recovery (%)
80	805.2	810.5	5.312	0.6598	100.7	[100.1 , 101.2]
100	1009	1013	4.548	0.4510	100.5	[100.0 , 100.9]
120	1207	1209	2.334	0.1934	100.2	[99.96 , 100.4]

Table 2: Trueness summary table from an Enoval report.

Amount level (%)	Mean introduced amount (mg)	Repeatability (RSD%)	Between-series (RSD%)	Intermediate precision (RSD%)
80	805.2	0.4988	0	0.4988
100	1009	0.4306	0	0.4306
120	1207	0.2234	0.05055	0.2291

Table 3: Relative precision summary table from an Enoval report.

5. Uncertainty

For accredited and medical laboratories operating under ISO 17025 & ISO 15189, reporting uncertainty is required. Enoval has a dedicated chapter on uncertainty, reporting the required statistics.

Concentration level (mg)	Mean introduced concentration (mg)	Standard uncertainty of the bias (mg)	Standard uncertainty (mg)	Expanded uncertainty (mg)	Relative expanded uncertainty (%)
80	805.2	1.640	4.338	8.675	1.077
100	1009	1.773	4.691	9.382	0.9302
120	1207	1.183	3.008	6.015	0.4983

Table 4: Uncertainty summary table from an Enoval report.

6. Accuracy (or Total Analytical Error) profile

Decide whether the method complies to the ATP: See in an eyewink which level you need to optimize, long before you enter the QC stage.

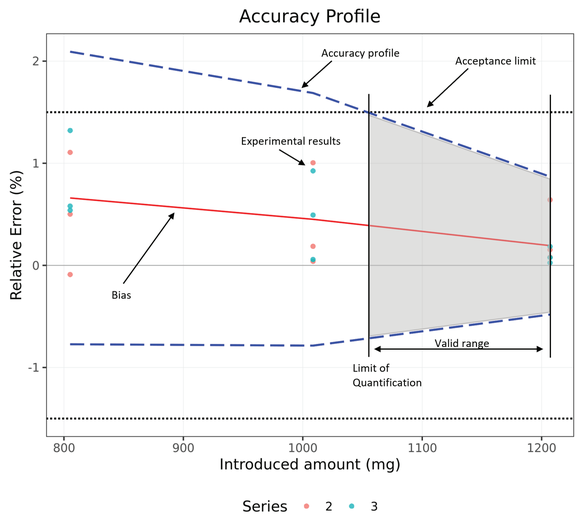

Figure 4: Accuracy profile (or Total Analytical Error profile) summarizing the analytical performance. The dotted black lines are the acceptance limits of ±1.5% as defined in the ATP. The red line is the interpolated expected bias. The blue dashed line is the interpolated β-expectation tolerance interval, i.e. the interval where we expect 95% of all our future measurements to fall. We see that when measuring at 800 mg and 1000 mg, more than the allowed 95% of measurements are expected to fall outside the acceptance limits.

7. Risk of falling out-of-specification

Understanding the risk associated with method performance deviations enables a proactive approach to quality management by identifying potential vulnerabilities or areas of improvement in the method.

Amount level (%)	Mean introduced amount (mg)	Beta-expectation tolerance limits (mg)	Relative Beta-expectation tolerance limits (%)	Risk (%)
80	805.2	[799.0 , 822.0]	[-0.7726, 2.092]	13.80
100	1009	[1001 , 1026]	[-0.7857, 1.688]	9.113
120	1207	[1201 , 1218]	[-0.4817, 0.8684]	2.754

Table 5: Summary of Total analytical error. β-expectation tolerance interval – i.e., the interval in which we expect 95% of all our future measurements to fall – is given in both absolute and relative terms. The Risk (%) is the expected probability (%) of any future measurement to fall outside the acceptance limit of ±1.5% (as stated in the ATP). As one can see the risk is higher than 100% - 95% = 5%, i.e., the maximal risk required by the ATP, when measuring at 800 and 1000 mg.

8. Linearity analysis

Verify whether the results (possibly back-calculated from the calibration) are in line with the true quantities.

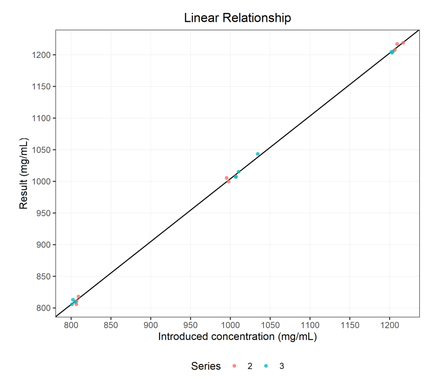

Figure 5: Linearity analysis graph: measured results versus the true quantity. Best linear fit using intercept (11.02) and slope (0.9940) with R^2 being 0.9998 and 95 % confidence intervals of the coefficients being [4.804, 17.23] for the intercept and [0.9880, 1.000] for the slope implying there is little reason *to not think* that was is measured, is the true quantity.

9. Writing an ICH Q2 compliant report in minutes

Enoval summarizes all these results, and more, into a comprehensive report in line with ICH Q2, ready for submission to the authorities, so that scientists do not have to spend days writing reports but can focus on developing methods.

10. Conclusion

Main advantages of SmartSTATS\Enoval:

- Standardize method validation reporting across the organization, etc.
- Up-to-date with latest authority requirements (EMA, FDA, etc.)
- Suitable for GxP use (GAMP5 validated, 21 CRF Part 11 compliant)
- SaaS: no maintenance cost for you (i.e. we maintain it for you)
- Reduces report writing to a couple of minutes instead of days
- Significantly reduce human errors
- Usable for method optimization within a QbD framework
- Increased statistical power due to use of one design for all validation criteria
- Going further than Excel (e.g. REML, quantile computation, etc.) to always give exact stat. results
- Easy to use: made by statisticians for non-statisticians
- Best-in-class decision making
- Can be integrated into existing LIMS or data platforms