A three-step workflow for microplate reader equivalency testing supporting European Pharmacopoeia guidelines

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Introduction

When a microplate reader method is transferred from one instrument to another, or sometimes one laboratory to another, it must be demonstrated that the new reader produces equivalent results. This is critical in regulated environments such as GMP and QC laboratories, where assay performance, product release decisions and historical data comparability rely on consistent analytical results. Equivalency testing provides documented evidence that any observed differences between microplate readers are not scientifically or statistically significant, and that the transferred method remains suitable for its intended use.

While the European Pharmacopoeia does not prescribe how to transfer methods between plate readers, it requires that equivalency be demonstrated using scientifically justified statistical approaches and predefined acceptance criteria.

This poster proposes a three-step evaluation plan aligned with European Pharmacopoeia Chapter 5.27.

Key features

- 3-step equivalency workflow between plate readers
- Statistics required by the European Pharmacopoeia
- Ready-to-use SoftMax Pro protocols with customized report and Pass/Fail results.
- Supports GLP and GMP compliance

Statistical approach and study design

Table 1 offers guidelines for evaluating reader performance characteristics using specific tests and predefined acceptance criteria to assess equivalence between two instruments. For quantitative comparisons of mean results from two readers, the approaches considered are relative difference and the two one-sided tests (TOST) procedure:

- < 6 replicates: relative difference is calculated and must fall within the predefined limit (typically 5–10%).
- ≥ 6 replicates: TOST demonstrate equivalence rather than absence of difference.

Additional guidelines: The assay is run on at least 2 occasions (2 days, 2 analysts) with n = 6 replicates per condition and read on both instruments. Reader comparison is made within-run; day and analyst serve as a robustness check.

Ready to use protocols (Figure 1) have been developed in SoftMax Pro with several tests outlined in Table 1 to help validated labs provide objective evidence that the analytical performance of the two readers is comparable, independently of routine assay variability.



Test 1: Validation plates

SpectraTest® ABS2, FL1, LM1 and Multi-Mode Validation Packages provide automated, comprehensive and traceable validation of the optical performance of Molecular Devices microplate readers. Run on both instruments (if compatible), they confirm that each reader operates according to its own specifications, so any difference observed in Tests 2 and 3 can be attributed to the method rather than to instrument performance. They verify linearity, dynamic range, precision and accuracy, alignment, signal to noise and signal to background, bias and repeatability. A reader failing any parameter must be serviced and re-qualified before Tests 2 and 3.



Test	Parameter used
Accuracy	Recovery, bias (%)
Precision	Repeatability (%CV)
Equivalence	TOST on final reportable results
Sensitivity	LOD / LLD / LOQ
Reportable result	Final back calculated concentration, potency, Recovery, ...
Curve quality	Lack-of-Fit (LOF)
Regression	Regression significance
Model quality	Residual
Curve characteristics	R^2, slope, intercept, EC_{50}, slope, top, bottom (4PL/5PL assays)

Table 1. *Examples of Reader Performances tests and Predefined acceptance criteria guidelines (Tests 2 and 3); values are examples to be tested and determined per method.*

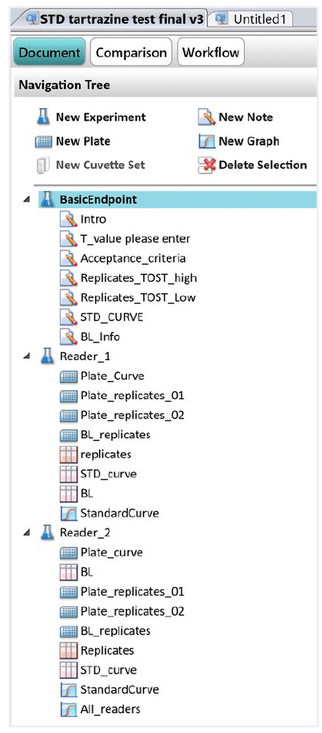


Figure 1. *Equivalency testing protocol for absorbance.*

Test 2: Specific dye linearity and sensitivity

A standardized reference material (tartrazine in absorbance, fluorescein in fluorescence, ATP in luminescence) generates a standard curve (Figure 2) and replicates at specific dilutions on both readers across the intended operating range. Several tests are used including linearity (R^2), precision (%CV) and sensitivity (LOD/LOQ) and are compared against predefined acceptance criteria (% difference or, TOST), with customized SoftMax Pro reports (Figures 3 and 4).

Test 3: Routine assay

The transferred method is run on both readers using representative samples, controls and standards. Final reportable results (back-calculated concentration, potency or recovery) are compared against predefined equivalence limits. Ready-to-use SoftMax Pro protocols can be configured with the tests in Table 1. If criteria are not met, the transfer is rejected pending investigation and corrective action.

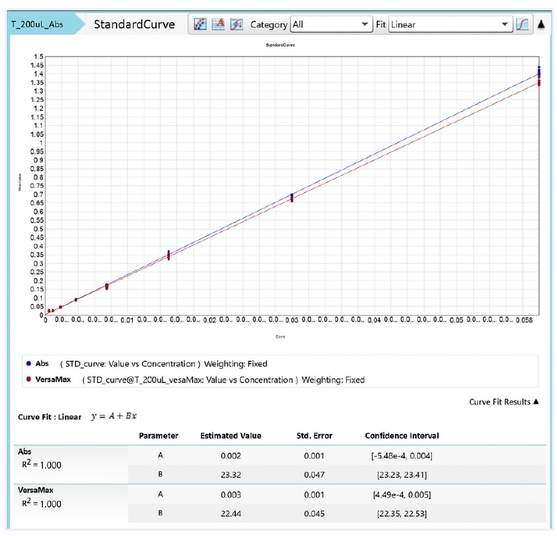


Figure 2. *Tartrazine standard curves using linear curve fit to show equivalence on two microplate readers.*

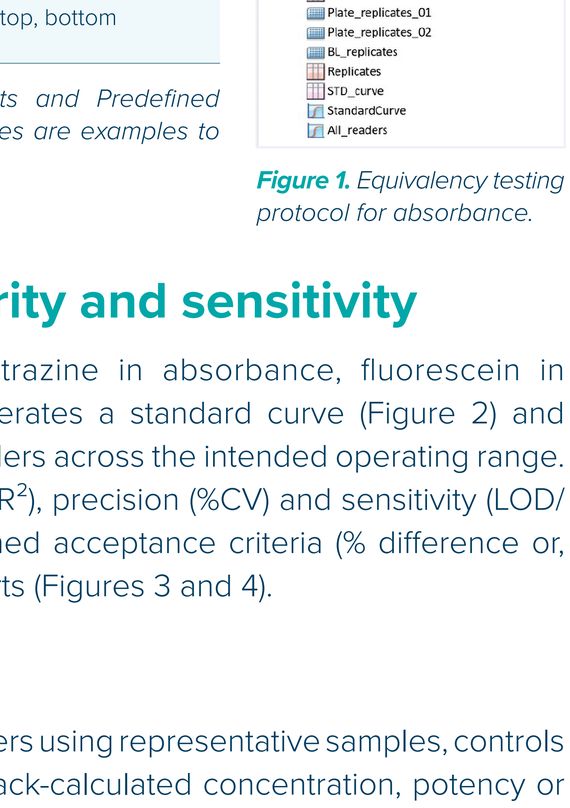


Figure 3. *Equivalency protocol Report for the standard curve.*

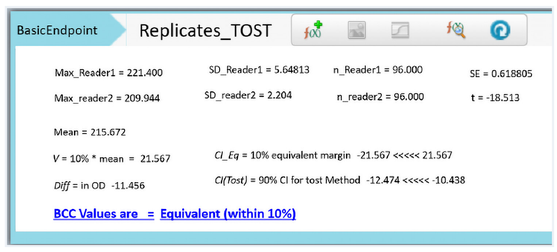


Figure 4. *Equivalency test TOST replicates.*

Conclusion

A structured three-step workflow — instrument validation with SpectraTest® plates, specific dye linearity and sensitivity, and the routine assay — provides documented, objective evidence that legacy and target microplate readers are equivalent. Predefined acceptance criteria and TOST satisfy European Pharmacopoeia expectations, while ready-to-use SoftMax Pro protocols automate curve fitting, statistics and pass/fail reporting for GLP/GMP method transfer.

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