Document «**AQC** EU-SANTE»

***A**nalytical **Q**uality **C**ontrol and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*

Document SANTE/11312/2021

Guidelines for Testing and Replacement of Standards in the AQC EU-SANTE Document

(SANTE/11312/2021: Section F – Testing and replacement of standards)

Antonio Valverde & Carmen Ferrer
Pesticide Residues Research Group & EURL-FV
University of Almería, Spain

This tutorial has been prepared on behalf of the EURL-FV

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- A.- Introduction and legal background
- B.- Sampling, transport, traceability and storage of samples
- C.- Sample Analysis
- D.- Identification of analytes and confirmation of results
- E.- Reporting results
- **F.- Pesticide standards, stock and calibration solutions**
- G.- Analytical method validation and performance criteria
- H.- Additional recommendations

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- Appendix A *(Method validation procedure: outline and example approaches)*
- Appendix B *(Examples of conversion factors)*
- Appendix C *(Examples for the estimation of measurement uncertainty)*
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Testing and replacement of standards

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F8 The stability of an existing and possibly expired «reference» standard may be checked by preparing a new stock standard and comparing the detector responses. The comparison should be undertaken using appropriate dilutions of individual standards or mixture of standards …

F9 The means from at least five replicate measurements for each of two solutions (old and new) should not normally differ by more than ± 10%. The mean value from the new solution is taken to be 100% and is also used as the basis for the calculation of the percentage-difference…

F10 The variability of (at least 5) replicate injections (expressed as repeatability-RSDr) should also be taken into account…

Old Stock Standard

C_{old}

nominal concentration (mg/L)

New Stock Standard

C_{new}

nominal concentration (mg/L)

F3 For the preparation stock standards not less than 10 mg of the «reference» standard should be weighed using a 5 decimal place balance.

Both (old and new) stock standard solutions are prepared with the same (or very similar) nominal concentration

Old Stock Standard

C_{old}

nominal concentration (mg/L)

Diluted Old Standard

New Stock Standard

C_{new}

nominal concentration (mg/L)

Diluted New Standard

Both stock standards are diluted in the same way

Old Stock Standard

C_{old}

nominal concentration (mg/L)

New Stock Standard

C_{new}

nominal concentration (mg/L)

Diluted Old Standard

Diluted New Standard

$A1_{old}$ $A2_{old}$ $A3_{old}$ $A4_{old}$ $A5_{old}$ $A1_{new}$ $A2_{new}$ $A3_{new}$ $A4_{new}$ $A5_{new}$

Five replicate injections of both diluted standards

(It is recommended to inject the old and new standards in alternating order)

$A1_{old}$ $A1_{new}$ $A2_{old}$ $A2_{new}$ $A3_{old}$ $A3_{new}$ $A4_{old}$ $A4_{new}$ $A5_{old}$ $A5_{new}$

$A1_{old}$	$A2_{old}$	$A3_{old}$	$A4_{old}$	$A5_{old}$	$A1_{new}$	$A2_{new}$	$A3_{new}$	$A4_{new}$	$A5_{new}$

Average of signals = A_{old}	Average of signals = A_{new}
RSD of signals = CV_{old}	RSD of signals = CV_{new}

F9 The means from at least five replicate measurements for each of two solutions (old and new) should not normally differ by more than +/- 10%.

F10 The variability of (at least 5) replicate injections (expressed as repeatability RSDr) should also be taken into account…

How to verify that the difference of the means is within ± 10% taking into account the RSD values?

$A1_{old}$	$A2_{old}$	$A3_{old}$	$A4_{old}$	$A5_{old}$	$A1_{new}$	$A2_{new}$	$A3_{new}$	$A4_{new}$	$A5_{new}$

Average of signals = A_{old}	Average of signals = A_{new}
RSD of signals = CV_{old}	RSD of signals = CV_{new}

The apropriate statistical test to verify that the absolute value of the difference of the means is less than a critical value (10%) is the «Two One-Sided *t*-Test (TOST)»

RESIDUES AND TRACE ELEMENTS

Testing the Accuracy of Analytical Standard Solutions Used for Quantitative Determination of Pesticide Residues

Ambrus et al. (2017) have found that, when the «Two One-Side *t*-Test (TOST)» is applied, there is a well defined linear relationship between the maximun value of the "pooled RSDs" **(Max CVp)** and the absolute value of the relative difference of the average values **(Δ_{rd} %)**. In case of 5 injections of both standards, at the 95% probability level, the following relationship is obtained:

Max CV_p = - 0.0086 Δ_{rd}% + 0.086

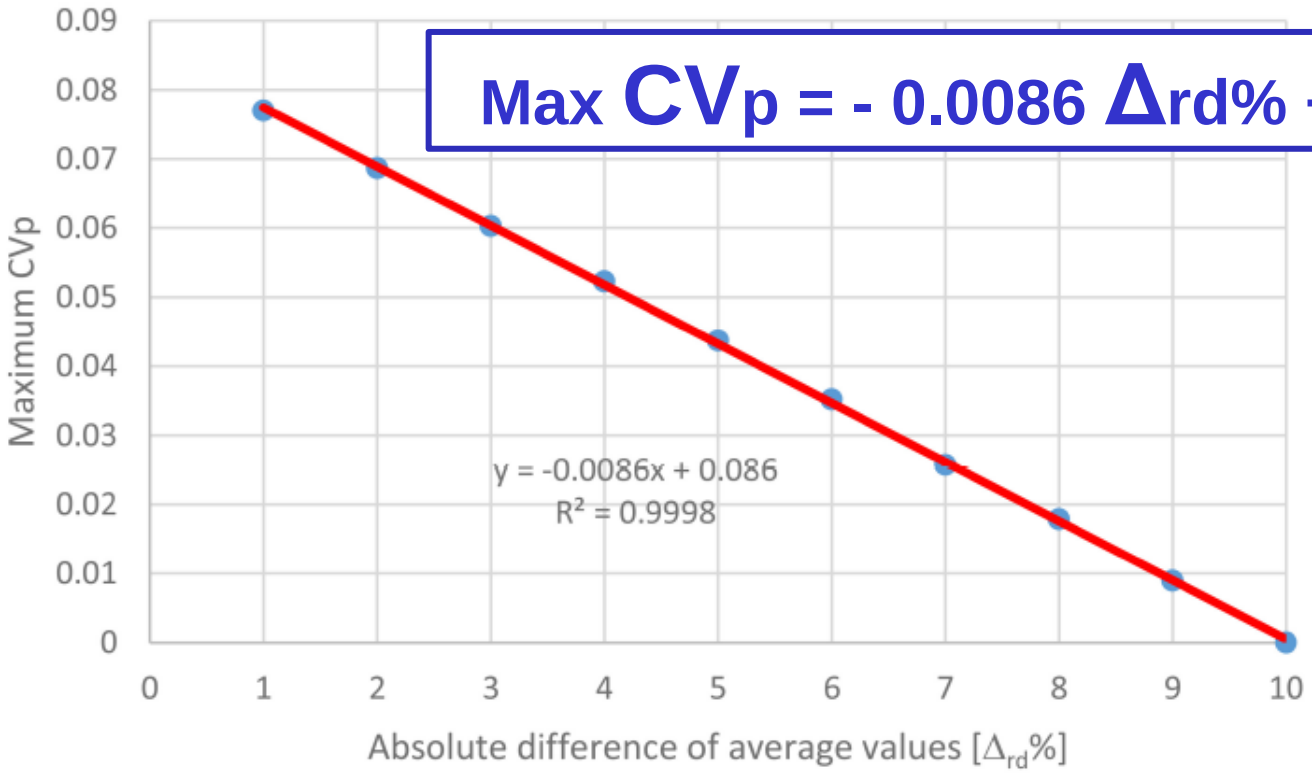

Figure 1. Relationship of Δ_{rd} to maximum CV_p, which can be used to verify that $\theta \leq \pm 10\%$ at the 95% probability level if $n_1 = n_2 = 5$.

$A1_{old}$	$A2_{old}$	$A3_{old}$	$A4_{old}$	$A5_{old}$	$A1_{new}$	$A2_{new}$	$A3_{new}$	$A4_{new}$	$A5_{new}$

Average of signals = A_{old}	Average of signals = A_{new}
RSD of signals = CV_{old}	RSD of signals = CV_{new}

Absolute value of the relative difference of average responses (Δ_{rd} %)

$$\Delta rd\% = |100*(A_{new} - A_{old})/A_{new}| = |100 - 100*A_{old}/A_{new}|$$

If the nominal concentrations of the stock standard solutions were not exactly the same, A_{old} must be multiplied by C_{new}/C_{old}

$A1_{old}$	$A2_{old}$	$A3_{old}$	$A4_{old}$	$A5_{old}$	$A1_{new}$	$A2_{new}$	$A3_{new}$	$A4_{new}$	$A5_{new}$

Average of signals = A_{old}	Average of signals = A_{new}
RSD of signals = CV_{old}	RSD of signals = CV_{new}

"Pooled RSDs" (CVp)

$$CVp = \left((CV_{old}^2 + CV_{new}^2)/2 \right)^{1/2}$$

The mean of the RDS values could be used for a very approximate calculus:

$$CVp \sim (CV_{old} + CV_{new})/2$$

If the point defined by the values obtained for "**Δrd%**" and "**CVp**" is above the acceptance criterion-line, **it cannot be stated**, with 95% probability, that the relative difference of means from five replicate measurements for each of two solutions (old and new) is within ±10%

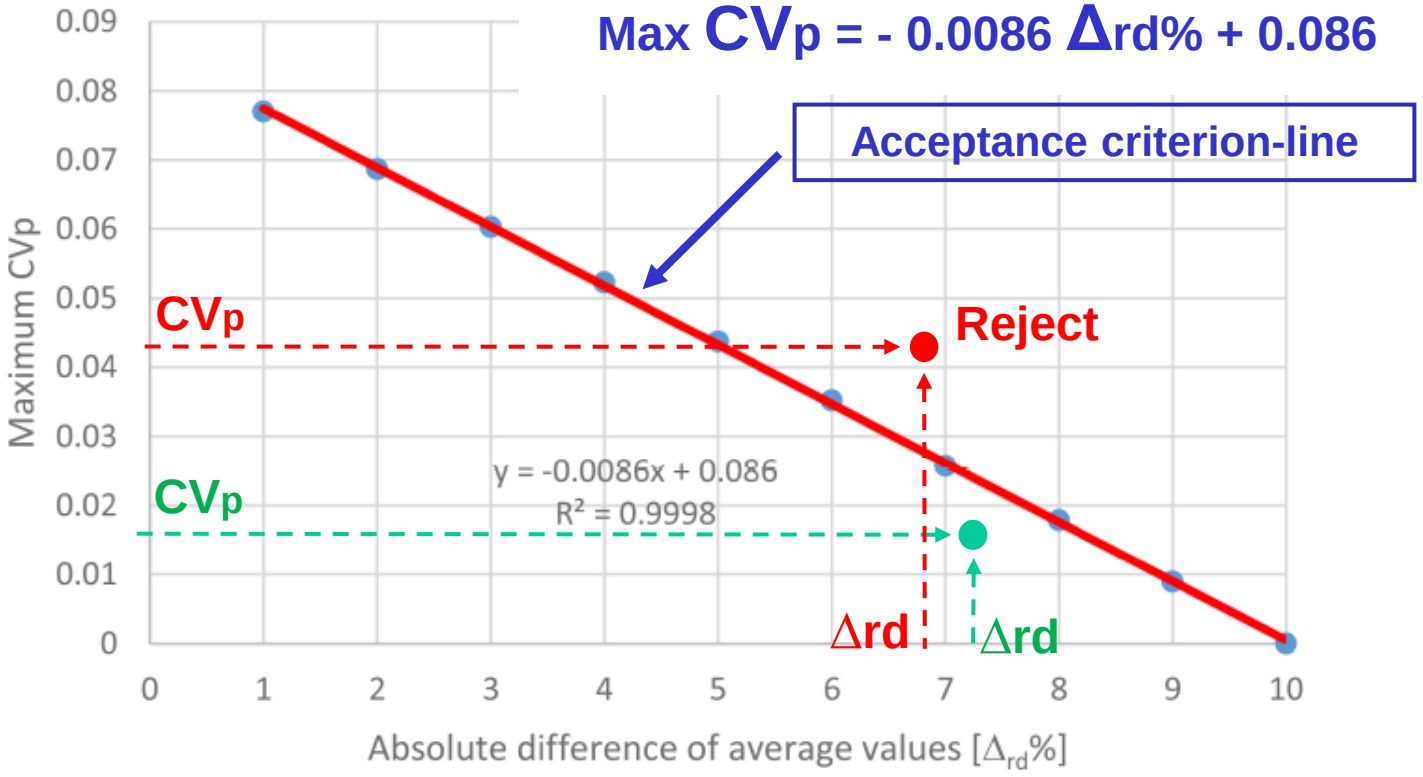

Figure 1. Relationship of Δ_{rd} to maximum CV_p, which can be used to verify that $\theta \leq \pm 10\%$ at the 95% probability level if $n_1 = n_2 = 5$.

Such as Ambrus et al. have pointed up, assuming that the typical repeatability CV of replicate injections into a LC-MS/MS system is around 2%, the maximun value permited for $\Delta_{rd}\%$ would be, in these cases, 7.67%

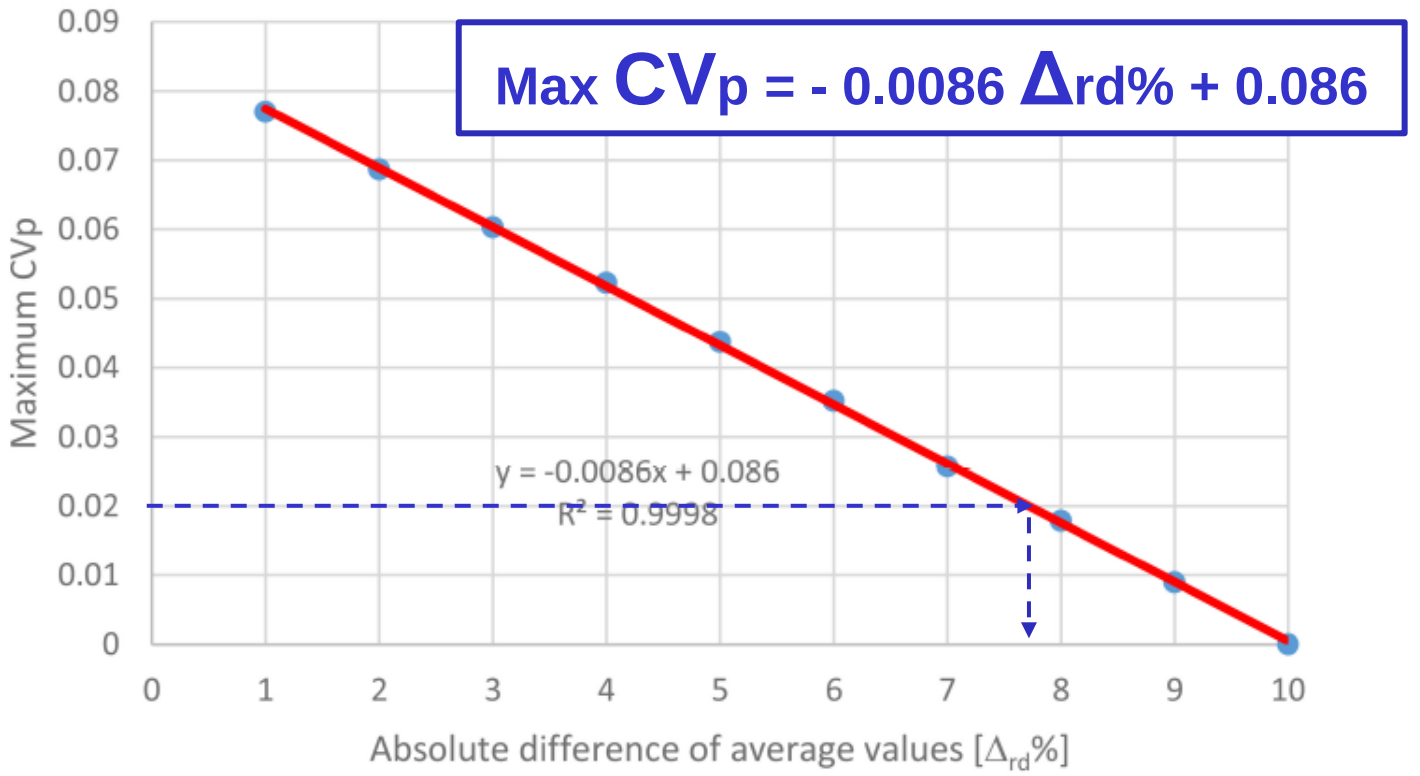

Figure 1. Relationship of Δ_{rd} to maximum CV_p, which can be used to verify that $\theta \leq \pm 10\%$ at the 95% probability level if $n_1 = n_2 = 5$.

CONCLUSION

To comply with the AQC EU-SANTE criteria for testing standards *(95% confidence and 5 replicate injections)*, the $\Delta_{rd}\%$ values must be lower than those indicated in the table:

Δ_{rd} (%)	CVp (%)
< 10	0
< 8.84	1
< 7.67	2
< 6.51	3
< 5.35	4
< 4.19	5
< 3.02	6
< 1.86	7
< 0.70	8

Max $CV_p = - 0.0086\ \Delta_{rd}\% + 0.086$

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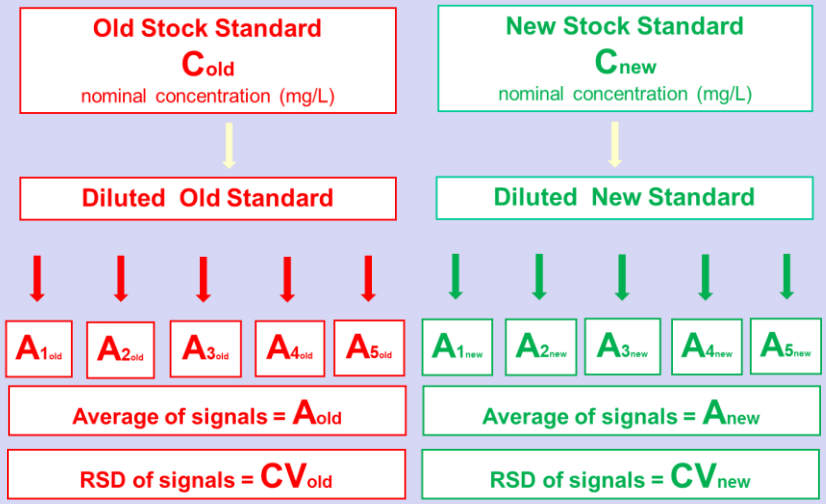
F9 The means from at least five replicate measurements for each of two solutions (old and new) should not normally differ by more than ±10%.



$$CV_p \sim (CV_{old} + CV_{new})/2$$

$$\Delta_{rd}\% = |100*(A_{new} - A_{old})/A_{new}| = |100 - 100*A_{old}/A_{new}|$$

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Many thanks for your attention!!!