Testing the accuracy of standard solutions used for quantitative determination of pesticide residues

Árpád Ambrus[1], Anikó Németh[2], Gabriella Suszter[2], Kamirán Á. Hamow[3], Etelka Solymosné Majzik[3]

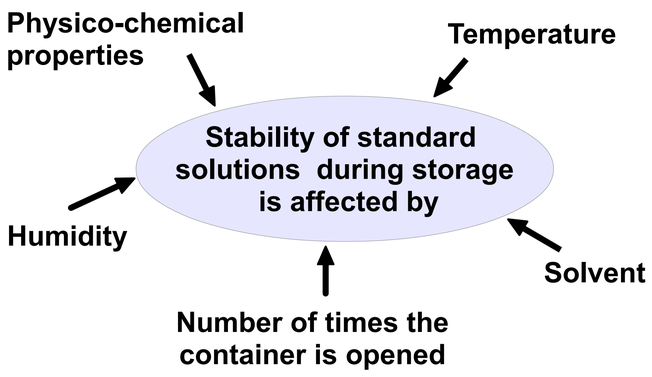
[1]National Food Chain Safety Office (retired); [2]Wessling Hungary Ltd., Budapest, Hungary; [3]National Food Chain Safety Office, Directorate of Plant Protection, Soil Conservation and Agri-environment, Pesticide Analytical Laboratory, Velence, Hungary

Stability - Accuracy

The stability of standard solutions during storage is affected by various factors. The accuracy of standard solutions should be tested in order to assure the quality of quantitative determination of pesticide residues.

Since the detector signals in GC-MS/MS and LC-MS/MS measurements can considerably vary depending on - for instance - condition of the ion source, contamination of the injector and column, etc., it is not possible to directly compare the detector signals aqcuired for a standard solution when it was freshly prepared and after a few months of application.

Therefore, it is necessary to develop **suitable statistical tests** for the evaluation of the accuracy of standard solutions.



Challenge and possible actions

Challenge: are the analyte concentrations in the standard mix accurate for quantitative determination of pesticide residues?

Case 1. The old standard mix is compared to a freshly prepared new standard mix.

Case 2. No new standard mix is prepared.

Case 3. New standard mix is prepared for the first time.

Case 1. The old standard mix is compared to a freshly prepared new standard mix

Are the analyte concentrations within the permissible ±10% in the "old" standard mix if the new standard mix is assumed to be correct?

Inject both old and new standards (e.g. 0.05 g/ml) 5 times ($n_1=n_2=5$)

Calculate the average responses (*relative to internal standard or a stable analyte*)

For example: $\bar{A}_{new}=1.00$; $\bar{A}_{old}=0.95$; $A_{\Delta}=0.05$; repeatability: $CV_{r,new}$; $Cv_{r,old}$

Method 1: testing equivalency of analyte concentrations in the two standard with two-sided t-test

Since the permissible deviation is ±10%, two-sided test shall be used

H_0: $\bar{A}_{new}=\bar{A}_{old}$
H_1: $\bar{A}_{new}\neq\bar{A}_{old}$

H_0 is accepted if $\mathbf{t_{calc}<t_{crit(0.05;df)}}$ for $n_1=n_2=5$; df = n-1; $t_{crit}=2.776$

$$t_{calc}=\frac{|\bar{A}_{new}-\bar{A}_{old}|}{S_p\sqrt{\frac{1}{n_{new}}+\frac{1}{n_{old}}}} \quad \text{(eq 1.)}$$

Calculate the pooled variance (S_p^2) from corresponding CV and average peak area (the standard deviation is: $S=CV_r \times \bar{A}$):

$$S_p^2=\frac{(n-1)\times(S_{new}^2+S_{old}^2)}{2n-2} \quad \text{(eq 2.)}$$

Table 1. Evaluation of the 2-sided t-test:

Analyte	$n_1=n_2$	CV_{new}	CV_{old}	$10^{-4}xS_p^2$	$\bar{A}_{new}$	$\bar{A}_{old}$	t_{calc}	H_0
1	5	0.022	0.019	4.05	1	0.95	3.93	Reject
2	5	0.016	0.015	2.23	1	0.92	8.47	Reject
3	5	0.072	0.055	38.7	1	0.92	2.03	Accept
4	5	0.008	0.009	0.655	1	0.91	17.6	Reject
5	5	0.072	0.075	48.2	1	0.89	2.51	Accept

Test outcome depends on S_p, the ±10% criterion cannot be verified!

Compare acceptance of H_0 and H_1 in Tables 1 and 2.

Method 1 is not applicable for testing equivalency of analyte concentrations.

Method 2: Apply two one-sided t-test (TOST)

Define acceptance criterion: θ=±10%

H_0: $\bar{A}_{new}-\bar{A}_{old}\leq\theta_{Lower}$ *or* $\bar{A}_{new}-\bar{A}_{old}\geq\theta_{Upper}$

H_1: $\theta_{Lower}<\bar{A}_{new}-\bar{A}_{old}<\theta_{Upper}$

Note the difference in null hypothesis!

The figure can be used for simply testing visually the „equivalency" of the two standard solutions by plotting CV_p as the function of $\bar{A}_{diff}$%. If CV_p is above the acceptance criterion line the concentrations of the tested compounds differ more than the ± θ (±10%) at 95% probability level.

The alternative hypothesis is proven at a specified level of confidence when the true difference in the mean values is within the boundaries specified by θ.
Calculate confidence intervals, CI, at 95% level:

$$CI=\bar{A}_{new}-\bar{A}_{old}\pm t_{0.1;\,(n-1)}\times\sqrt{s_p^2\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} \quad \text{(eq 3.)}$$

If CI is within *θ,* the difference is within acceptance criterion (±10%, ±0.1)

Table 2. Evaluation of TOST test:

Analyte	$n_1=n_2$	CV_{new}	CV_{old}	$10^{-4}xS_p^2$	$\bar{A}_{new}$	$\bar{A}_{old}$	CI^+	CI^-	H_1
1	5	0.022	0.019	4.05	1	0.95	0.074	0.027	Accept
2	5	0.016	0.015	2.23	1	0.92	0.100	0.060	Accept
3	5	0.072	0.055	38.7	1	0.92	0.164	-0.004	Reject
4	5	0.008	0.008	0.585	1	0.91	0.100	0.080	Accept
5	5	0.072	0.075	48.2	1	0.89	0.204	0.016	Reject

Alternatively an automated excel template (that contains Table 2. and a quick test for CV_p) might also be used to evaluate the accuracy of standard mixtures. (Download through QR)

Note: The closer the difference to *θ* the smaller the CV_p must be.

If the difference is larger than 10% the H_1 will be rejected regardless the CV of repeated injections!

Case 2. No new standard mix is prepared

Method 3: checking the stability of components in currently used "old" standard mix.

Retrieve the relative signals of the daily replicate injections (e.g. the last two in each injection sequence) of the standard mix when it was new ($A_{i,new}$) and during current use (now it is "old", $A_{i,old}$), for the last N ≥10 sequence (≥ response pairs), respectively.

Calculate the average within day relative repeatability of injections of i^{th} component (CV_i) if A_{ib} and A_{ie} are the relative responses at the beginning and end of each of the N sequences, or the last two standard injections in long sequences, where the relative responses might shift:

$$\overline{CV}_{i,old}=\frac{1}{1.128N}\times\sum_{j=1}^{j=N}\left(\frac{|A_{ib,j}-A_{ie,j}|}{\bar{A}_{i,j}}\right) \quad \text{(eq.4).}$$

Calculate also $\overline{CV}_{i,new}$ with equation 4.
Calculate the average responses $\bar{A}_{i,new}$ from the 2N injections of "new" standard and from the 2N injections of "old" standard $\bar{A}_{i,old}$
Insert the CV and average areas in the template (Table 2) for the calculation of CI with equation 3.

If CI is within *θ*, the standard solution can be further used, if not prepare a new standard mix and compare the analyte concentrations in the old standard mix with those of freshly prepared ones.

Case 3. New standard mix is prepared for the first time

If a standard mix is prepared for the first time, its accuracy can only be verified if a second mix is prepared independently, and the equivalency of the two standard mix is tested with TOST applying a more stringent criterion (e.g. 5%) than for testing the old standard mix.

Conclusions and recommendations

1. Testing the accuracy of nominal concentrations of components of standard mixes containing several hundreds of components is a tedious, time consuming procedure, but it is necessary for obtaining accurate results.

2. The presented calculations are correct only if equal number of data are available for the components of the two standard mixtures, but **n** can be different for different analytes.

3. For testing if the concentrations of the analytes in standard mixtures are **within the ±10% criterion, Method 2 (TOST) shall be used** and not the two-sided t-test!

4. **Method 3** can be used as a **quick screening test** without the need for preparing new standard mix. However, if $\bar{A}_{i,new}-\bar{A}_{i,old}>\theta$, verification of compliance with the specified criterion can only be done with TOST (Method 2).

5. The performance of the tests can be facilitated if the responses retrieved are automatically entered in the relevant Excel template containing the calculation formula. This procedure depends on the data acquisition software.

6. Application of accurate standard mixes can be improved (if the applicable solvents permit), by **including as many known instable compounds into one sub-mix** as possible to limit the number of sub-mixes that shall be discarded and freshly prepared.

7. Include potential degradation products of analytes in different sub-mixes to facilitate their detection if sub-mixes are tested separately.

8. If a standard mix is prepared for the first time, **two standard mixes shall be prepared independently**. If the concentrations in the two standard mixes are within the acceptance criterion, the two mix can be combined and the average concentrations of the components can be used for quantifying the residues in samples. If the difference is larger, a case by case decision is required on the use of standard solutions.