Workflow to perform quantification by standard addition procedure

(Last update 1 July 2017)

Standard addition is an effective way to compensate for low recoveries and/or matrix effects. When additions are done to extract aliquots the procedure will only correct for matrix effects. This approach is often used in cases where matrix effects are very strong and no blank matrix is available for preparing a suitable external matrix matched calibration. For this approach it is important to know how much sample is contained in the extract aliquot used. If the additions are done to sample portions, prior to extraction, the procedure will compensate for both low recoveries and matrix-effects. This approach is often used when the analyte of interest shows an unacceptably low recovery (large bias). Because of linear extrapolation linearity of response throughout the measured concentration range is paramount. Furthermore it should be considered, that added amounts are not too small in order to reduce the influence of measurement variability on the slope of the linear regression.

For the **standard addition to extract aliquots** the following four steps should be followed:

1) Estimate the approximate concentration of the analyte in the sample, e.g. using external calibration.
Example: Chlorpyrifos concentration ~ 0.2 mg/kg

2) Calculate the absolute amount of the analyte in a representative volume of the sample extract, for example 1 ml.
Example: Extract concentration 1 g/mL (typically for QuEChERS extracts of fruits and vegetables) → ~ 0.2 µg in 1 mL sample extract.

3) Take 4 vials and fill 1 mL of sample extract in each vial. Only 3 of the 4 aliquots are spiked. The amounts of analyte added should be similar to the expected amount of analyte in the aliquots e.g. 0.5x, 1x and 1.5x the estimated amount. The linearity range allowing it, greater amounts may be added (e.g. 1x, 2x and 3x the estimated amount).

4) Prepare a suitably concentrated standard solution so that the smallest analyte amount to be added is contained in 50 or 100 µL.
Example: Addition levels 0.1 µg, 0.2 µg, 0.3 µg. Prepare a standard solution of chlorpyrifos at 1 µg/mL; 100 µL of this solution contains 0.1 µg.

5) Spike the vials 2 - 4 with the appropriate volume of the pesticide standard solution; in vial 1 the original sample remains without addition of standard solution. Adjust the volume by adding the corresponding solvent amounts.

In case of a suspected MRL violation it is recommended to re-extract the sample in duplicate and to conduct a standard addition experiment for each. In parallel a recovery experiment using the same or a similar matrix is to be conducted and the extract also subjected to a standard addition procedure. The spiking levels should be chosen to be similar to that of the sample suspected to exceed the MRL.

Example 1: Residues of Chlorpyriphos in Sweet Pepper, concentration ~0.2 mg/kg

Standard addition TO EXTRACT ALIQUOTS (exemplary pipetting scheme)				
Extract aliquot volume used		1 ml	**Commodity:** Sweet Pepper **Analyte:** Chlorpyrifos **Expected Conc.**: 0.2 mg/kg	
Sample amount represented in the aliquot		1 g		
Expected absolute amount of pesticide in the aliquot		~0.2 µg		
Conc. of chlorpyrifos std solution to be used for StAdd		1 µg/mL		
	no addition	**addition level 1**	**addition level 2**	**addition level 3**
Volume of sample extract aliquots	1000 µl	1000 µl	1000 µl	1000 µl
Added volume of an appropriate dilution of pesticide stock solution	-	100 µL	200 µL	300 µL
Resulting mass of pesticide added to each vial	0	0.1 µg	0.2 µg	0.3 µg
Volume of solvent to be added	300 µL	200 µL	100 µL	-
Final volume	1300 µL	1300 µL	1300 µL	1300 µL

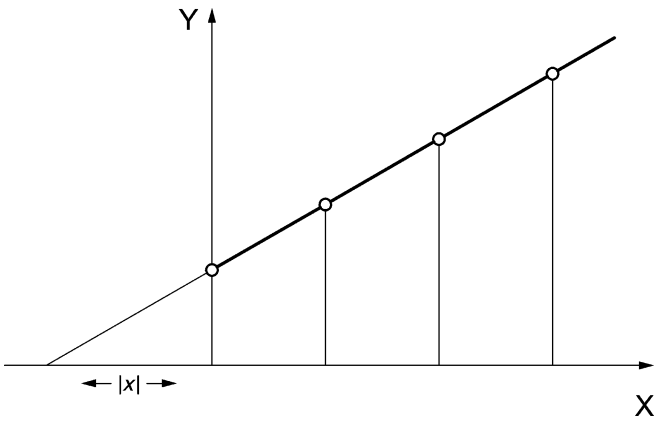
Example 2: Residues of Methamidophos in tea, concentration ~2 mg/kg
(Extraction with QuEChERS-Method (2 g sample + 10 ml water+10 ml MeCN))

Standard addition TO EXTRACT ALIQUOTS (exemplary pipetting scheme)				
Extract aliquot volume used		1 ml	**Commodity:** Tea **Analyte:** Methamidophos **Expected Conc.**: 2 mg/kg	
Sample amount represented in the aliquot		0.2 g		
Expected absolute amount of pesticide in the aliquot		~0.4 µg		
Conc. of methamidophos std solution to be used for StAdd		2 µg/mL		
	no addition	**addition level 1**	**addition level 2**	**addition level 3**
Volume of sample extract aliquots	1000 µl	1000 µl	1000 µl	1000 µl
Added volume of an appropriate dilution of pesticide stock solution	–	100 µL	200 µL	300 µL
Resulting mass of pesticide added to each vial	0 µg	0.2 µg	0.4 µg	0.6 µg
Volume of solvent to be added	300 µL	200 µL	100 µL	- µL
Final volume	1300 µL	1300 µL	1300 µL	1300 µL

Example 3: Residues of Cyromazine in Lettuce, conc. determined in initial analysis 0.06 mg/kg (expected recovery rate ~ 30%), thus expected conc. in sample ~ 0.2 mg/kg

Standard addition TO SAMPLE PORTIONS (exemplary pipetting scheme)				
Size of sample portion		10 g	**Commodity:** Lettuce	
Expected absolute amount of pesticide in the sample portion		~2 µg	**Analyte:** Cyromazine	
Conc. of Cyromazine std solution to be used for StAdd		10 µg/mL	**Expected Conc.**: 1 mg/kg	
	no addition	**addition level 1**	**addition level 2**	**addition level 3**
Volume of sample extract aliquots	1000 µl	1000 µl	1000 µl	1000 µl
Added volume of an appropriate dilution of pesticide stock solution	–	100 µL	200 µL	300 µL
Resulting mass of pesticide added to each vial	0	1 µg	2 µg	3 µg
Volume of solvent to be added	300 µL	200 µL	100 µL	- µL
Final volume	1300 µL	1300 µL	1300 µL	1300 µL

Quantification using the procedure of standard addition, schematically



Key

Y Ratio of peak area of analyte divided by peak area of ISTD

X Added absolute mass of analyte $m_{pest}^{std\ add}$ in µg

|x| absolute amount of analyte in the sample portion (in µg) before standard addition (y = 0)

$$x = \frac{y - intercept\ (c)}{slope\ of\ the\ curve\ (b)}$$

$$Conc.\ of\ analyte\ in\ sample = \frac{x}{Amount\ of\ sample\ in\ spiked\ aliquot}\ [\mu g/g] = [mg/kg]$$