

# Combining standard addition with blank addition

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## Introduction

The standard addition method is a well-known approach to quantification in analytical chemistry. It is used in order to circumvent systematic errors that are due to the sample preparation procedure or are caused by matrix effects.

Standard addition involves adding varying amounts of the analyte to sample portions of fixed mass or fixed volume and submitting those portions to the sample preparation procedure. After measuring the final extract solutions, the observed signals are linearly regressed on the spiked amounts. The original unknown amount is estimated by the opposite of the abscissa intercept of the fitted straight line. A limitation of this method is that only data points with abscissa values equal to and greater than zero are available so that there is no information on whether linearity holds below the spiking level zero. To overcome this limitation, standard addition can be combined with blank addition [1]. Blank addition means that defined mixtures of blank matrix and sample material are subjected to sample preparation to give final extract solutions. This contribution presents the use of the combined approach to quantify nandrolone in a male bovine urine sample.

## Method

For derivation of formulae and a description of the properties of the combined approach, see [1]. Suppose that a liquid sample contains a particular compound of interest at unknown concentration. To determine this concentration,  $n$  aliquots of the sample are spiked with the analyte at concentration levels  $(x_i)_{i=1,\dots,n}$ . Furthermore, sample material is diluted with blank matrix to give mixtures with sample volume fractions of  $(k_j)_{j=1,\dots,m}$  (ratios of sample volume to volume of total mixture). From each solution of the two series an aliquot of the same volume is taken and submitted to chemical analysis. On the basis of the data, the estimate for the ordinate intercept ( $b_0$ ), the estimate for the slope ( $b_1$ ), the estimate for the originally present unknown concentration ( $\hat{x}^*$ ) and the abscissa values of the blank addition points ( $\hat{x}_j$ ) can be computed as follows:

$$b_0 = \frac{\sum_i x_i^2 \left( \sum_i y_i \sum_j y_j k_j \right) - \sum_i x_i y_i \sum_i x_i}{\sum_i x_i^2 \left( n + \sum_j k_j^2 \right) - \left( \sum_i x_i \right)^2}$$

$$b_1 = \frac{\sum_i x_i y_i \left( n + \sum_j k_j^2 \right) - \sum_i x_i \left( \sum_i y_i \sum_j y_j k_j \right)}{\sum_i x_i^2 \left( n + \sum_j k_j^2 \right) - \left( \sum_i x_i \right)^2}$$

$$\hat{x}^* = \frac{b_0}{b_1}, \quad \hat{x}_j = (k_j - 1) \hat{x}^*$$

$y_i$  denotes the  $i$ th standard addition signal and  $y_j$  denotes the  $j$ th blank addition signal.

## Example case

A male bovine urine sample was analysed for its content of the steroid nandrolone by an LC-MS assay. A series of six aliquots of the urine sample were spiked with the target analyte at levels of 0, 0.5, 1.0, 1.5, 2.0 and 2.5  $\mu\text{g/L}$ . Additionally, dilutions of the urine sample with nandrolone-free urine were prepared at sample volume fractions of 0.1, 0.3, 0.5, 0.7 and 0.9. Table 1 lists the observed signals, the estimated parameters, the estimate for the originally present unknown concentration, the 0.95 confidence interval for the originally present unknown concentration (for formula, see [1]) and the calculated abscissa values of the blank addition data points. Figure 1 displays the data points, the straight line fitted to the combined data set and the 0.95 confidence bands.

Table 1: Quantification of nandrolone in a bovine urine sample (all concentrations in  $\mu\text{g/L}$ ).

$x_i$	$y_i$	$k_j$	$y_j$	$b_0$	$b_1$	$\hat{x}_j$	$\hat{x}^*$	0.95 confidence interval
0.0	5394708	0.1	464139	4950060	2696536	-1.65	1.84	[1.62; 2.08]
0.5	6061705	0.3	1497300			-1.29		
1.0	7872740	0.5	2492913			-0.92		
1.5	8822356	0.7	3069726			-0.55		
2.0	10548385	0.9	4348496			-0.18		
2.5	11587569							

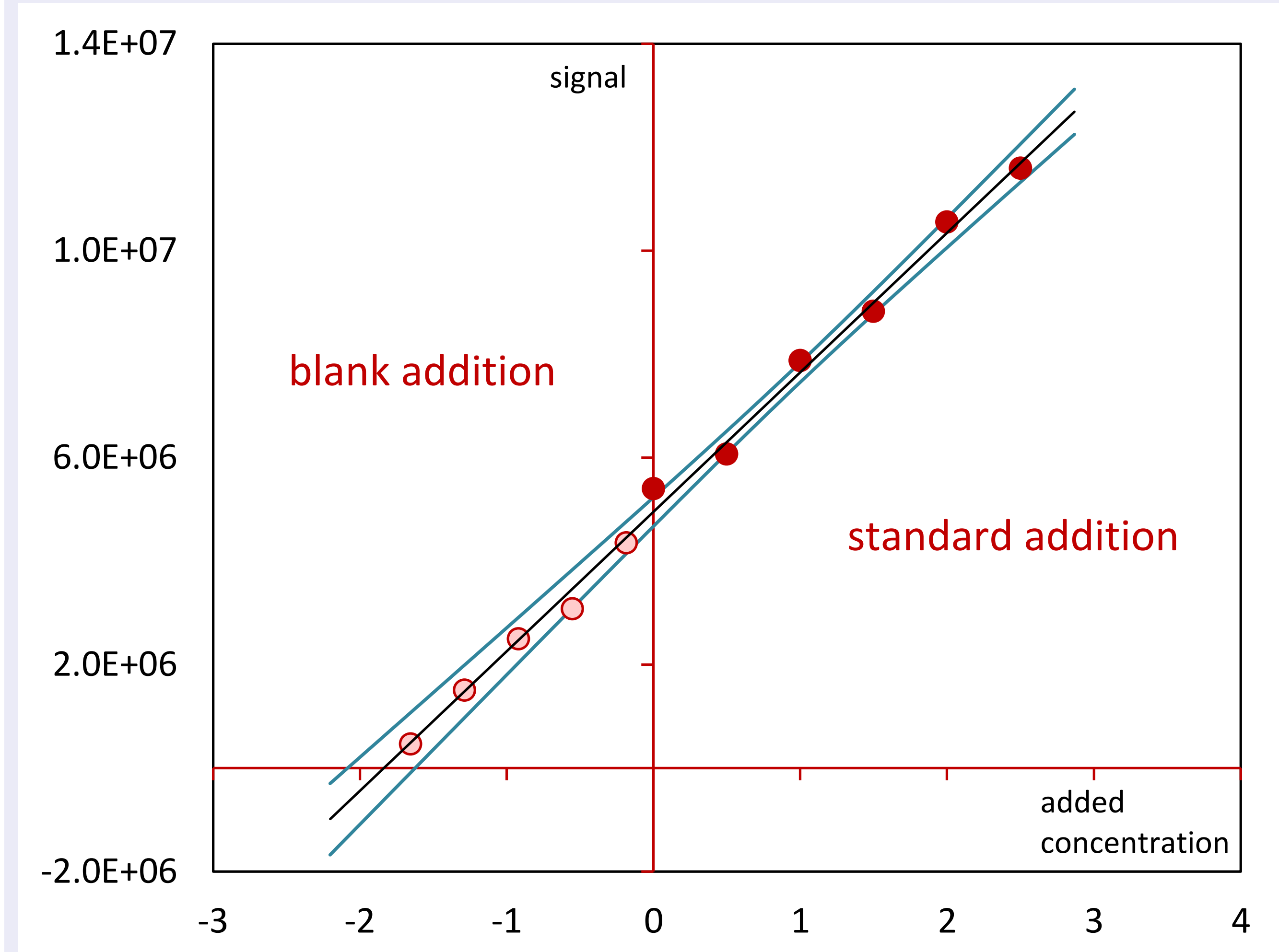


Figure 1: Quantification of nandrolone in a bovine urine sample by standard addition combined with blank addition (plot of the standard and blank addition line, the 0.95 confidence bands and the data points).

## References

[1] Steliopoulos P (2015) *MethodsX* 2:353-359.