APPLICATION OF THE 'ACCURRACY PROFILE' CONCEPT FOR THE EVALUATION OF THE CALUX METHOD AS QUANTITATIVE ANALYTICAL METHOD

I.Van Overmeire, L. Goeyens, S. Carbonnelle, V. Draguet, J. De Beer

Scientific Institute of Public Health, J. Wytsmanstraat 14, B-1050, Brussels, Belgium

Introduction

The quality of the results obtained with an analytical procedure is of importance for laboratories under accreditation. In general, the validation of an analytical procedure yields several validation parameters which indicate the performances of the method. However, the evaluation of the capacities of analytical methods can sometimes be confusing because there are several methods in use for the determination and definition of the same performance characteristic. Moreover, a general validation protocol is lacking. Here we present and illustrate a validation approach to unambiguously demonstrate the fitness of the method for its intended use.

Objectives

The aim of this paper is to explain the concept and applicability of the 'accuracy profile'. This concept has already been described by members of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP). With this approach it can be decided whether the method is valid for measuring results that fall with a known probability β inside predefined acceptance limits λ.[1,2] As an illustration, a practical example is presented, using quantitative data obtained with the CALUX assay.

Materials and methods

Each analytical method is characterised by a bias (systematic error δ_M) and a precision (random error σ_M, measured by a standard deviation or variance).
The terms accuracy, bias and trueness are used according to the ISO-definitions.
The β-expectation tolerance interval is calculated according to references 2 and 3 and the applied formula contains the following parameters: theoretical concentration value μ, estimate of the bias and the standard deviation of intermediate precision, trueness. Additionally, the probability (or risk) for future results to fall outside the acceptance limits is integrated in the tolerance interval:

$$\hat{\delta}_M \pm Q_t \times k_s \hat{\sigma}_M$$

with $\hat{\delta}_M$ = bias estimate; Qt = quantile of Student t distribution with ν degrees of freedom;
$\hat{\sigma}^2_M = \hat{\sigma}^2_W + \hat{\sigma}^2_B$ = intermediate precision variance estimate (within and between series variance).

Experimental data were obtained with the CALUX bioassay using the mouse hepatoma H1L6.1 cell line from Xenobiotic Detection Systems (Durham, NC, USA) as previously described.[4]

Results and discussion

- *Concept of β-expectation tolerance interval and accuracy profile[3]:*

The basic idea behind this concept is that analysts expect an analytical procedure to return a result x which differs from the unknown 'true value' μ of the analysed sample by less than an acceptability limit λ. This requirement is expressed by equation 1:

$$-\lambda \langle x-\mu < \lambda \Leftrightarrow |x-\mu| < \lambda \qquad (1)$$

The acceptability limit λ can be freely chosen depending on the objective of the analytical procedure.

The ultimate objective of the validation is to guarantee that most of the measurements the procedure will provide in the future are accurate enough-close enough to the unknown true value of the sample assayed.
In other words it must be very likely that the difference between every measurement x of a sample and the true value μ will fall inside the predefined acceptability limits λ.
This is translated in equation 2:

$$P(|x-\mu| < \lambda) \geq \beta \qquad (2)$$

where β is the probability that a measurement falls inside the the acceptability limits defined by λ.

β-expectation tolerance interval is an interval which contains an expected proportion β of future results for a given bias and standard deviation.

$$E_{\mu M, \sigma M} \{P [|xi-\mu| \delta_M, \sigma_M]\} > \beta$$

With E the 'expected value' of the result for a given bias and standard deviation.

The calculation involves estimating the bias and the standard deviation of intermediate precision of the method (cfr supra).

This interval can be used as decision rule to accept a procedure as valid. i.e. a procedure is accepted as valid if, according to the bias and precision observed during the validation experiments, the proportion of measurements inside the acceptability limits λ is greater or equal to β (β is chosen a priori).
When this interval is extended to a whole range of expected measurements it is called the *accuracy profile of the analytical procedure (Figure 1):*
The accuracy profile is constructed for a given set of concentration levels by using the β-expectation tolerance interval for each concentration level with respect to the acceptance limits and the relative bias (100 .(x-μ)/μ):
For each concentration level estimates of bias and precision are obtained and β-expectation tolerance limits are calculated. The upper and lower tolerance limits are then connected by straight lines (interpolating lines) .For certain concentration levels the tolerance level can be wider than the acceptance limits. The limits of quantification are at the intersection between the interpolating lines and the acceptance limits. Below the lower limit of quantification (LLQ) or above the upper limit of quantification (ULQ) it is unacceptable to say that the procedure accurately quantifies the analyte. The LLQ is the smallest quantity of the substance that can be measured with a given trueness and precision. The accuracy profile integrates in a single graph (Figure 1) all the elements essential for the validation, i.e. the bias, the precision, the risk and the quantification limits.

- *Application of concepts*

This concept is illustrated using validation data obtained with the CALUX bioassay for the matrix milk (see Figure 2). In this case we used validation data obtained for enriched milk samples at 3 concentration levels (1; 3 and 6 pg TEQ/g fat). To obtain estimates of between and within series variances the experimental design of the validation was chosen in order to obtain for each concentration level 2 or 3 replicates and this for 3 different series. Each series of analyses was performed on different days. The acceptability limit λ was set to 30 % (in

accordance with the maximal allowed rsd (relative standard deviation) of 30 % stated in EU requirements for screening methods used for dioxin determinations.[5]

Figure 1

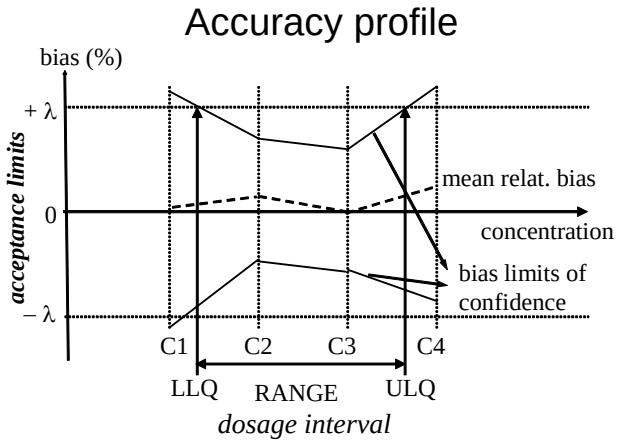
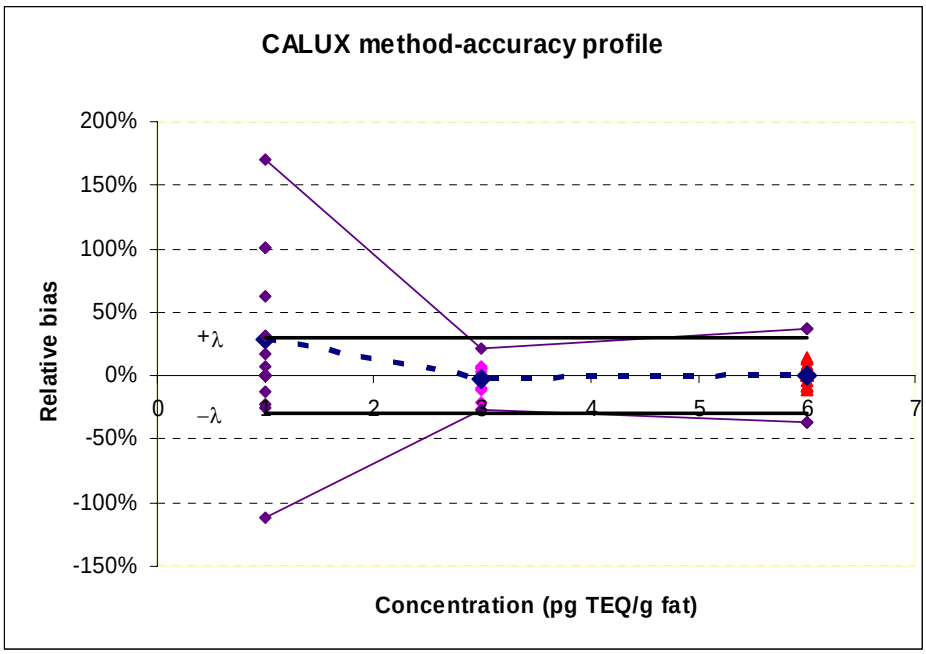


Figure 2



However, it must be clear that the relative standard deviation and the relative bias of the method are completely different parameters, the first one expressing the random error and the latter one the systematic error of the method. The tolerance interval shown in Figure 2 was calculated for β = .0.9 or 90 % certainty that results will be within acceptance limits.

The method can be considered accurate at β probability level, for the concentration level in question if the tolerance interval is included within the limits defined a priori, according to the method objectives.
The example in Figure 2 illustrates that the CALUX method satisfies for concentrations between 3 and 6 pg TEQ/g fat. This concentration range can be considered as the accuracy profile for this application. It is the range where the procedure is expected to quantify at least a proportion β of the samples with a predefined accuracy. Below 3 pg TEQ/g fat the β expectation tolerance interval falls outside the tolerance limit that was defined for the method. This means that below 3 pg TEQ/g fat less than 90 % of future results will be within the acceptance limits.
The accuracy profile is an alternative approach that allows a visual evaluation of the ability of the procedure to fit its purpose.

The accuracy profile was also determined using validation data obtained with the CALUX method for the matrix eggs. This indicated a difference with the milk data. A larger tolerance interval for similar concentrations tested was observed, indicating a matrix effect (not shown). However, this exercise was conducted using a preliminary data set and the acquisition of more validation results should lead to better results.
The accuracy profile also depends on the risk one is willing to take.
For example, accepting a risk of 10 or of 5 % (or β = 0.8 or 0.9 respectively) will result in a different tolerance interval. The β-expectation tolerance interval becomes smaller for a lower β value.

As validation protocol we propose to collect data for at least 3 concentration levels. The minimum number of results per concentration level should be: 3 x 3 (3 replicates on 3 different occasions).

In conclusion, the concept of 'accuracy profile' can be considered as an integrated validation approach.
An easy visual determination of the LOQ and a demonstration for which concentrations the method fulfils to the acceptance limits is possible.

References

1. Commission SFSTP, Hubert P, Nguyen-Huu J, Boulanger B, Chapuzet E, Chiap E, Cohen N, Compagnon P, Dewé W, Feinberg M, Lallier M, Laurentie M, Mercier N, Muzard G, Nivet C, Valat L.*STP Pharma pratiques* 2006 ;13 :101-138.
2. Commission SFSTP, Hubert P, Nguyen-Huu J, Boulanger B, Chapuzet E, Cohen N, Compagnon P, Dewé W, Feinberg M, Laurentie M, Mercier N, Muzard G, Valat L.*STP Pharma pratiques* 2006 ;16 :28-58.
3. Feinberg M, Boulanger B, Dewé W. *Anal Bioanal Chem* 2004 ;380 :502-514.
4. Van Overmeire I, Van Loco J, Roos P, Carbonnelle S, Goeyens L. *Talanta* 2004;63:1241-1247.
5. Commission Directive 2002/69/EC. *Off J Eur Comm* 6.8.2002, L209/5.