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Letter to the Editor

## Response to comments on "Uncertainty profiles for the validation of analytical methods"

This manuscript presents a response to a recently published article in *Talanta* 88 (2012) 769–771 by Rozet et al. [1] in which the authors comment on some aspects of our previous work [2] entitled "Uncertainty profiles for the validation of analytical methods" *Talanta* 85 (2011) 1535–1542. We present here new arguments to support our previous findings and to clarify some aspects regarding to the use of the β-content, γ-confidence tolerance interval for share validated analytical methods and to estimate the measurement uncertainty.

First, we noted that Rozet et al. [1] commented on our choice of using the β-content, γ-confidence tolerance interval and justified their choice to use the β-expectation tolerance by the fact that the latter is equivalent to the prediction interval and therefore it can predict the future measurements. As a result, the β-expectation tolerance can be used as decision tools for determining the validity of an analytical method since this interval is able to predict the results generated by the method in routine phase.

Indeed, to say in the absolute, that the β-expectation tolerance interval is always equivalent to the prediction interval is not true. The key difference is thus to distinguish prediction and tolerance.

### 1. When β-expectation tolerance intervals = prediction intervals

For models with one variance component a β-expectation tolerance interval and a prediction interval are equal. For these models it is assumed that $R = 0$ (the ratio of between to within subject variance) and no degrees of freedom adjustment is needed. In this case, the formula for a β-expectation interval reduces to the formula for a prediction interval [3–6].

The same relationship between β-expectation tolerance intervals and prediction intervals does not exist when there is more than one variance component in the model [7,8].

### 2. When β-expectation tolerance intervals ≠ prediction intervals

For models with two variance components the β-expectation tolerance interval is different from prediction interval and this difference is caused by different degrees of freedom used to compute the two estimates.

The interval estimates are equal if the degrees of freedom for a β-expectation tolerance interval are adjusted to equal the degrees of freedom for a prediction interval. For models with more than one variance component prediction intervals tend to be wider than β-expectation tolerance intervals (for example, the length of a β-expectation interval is 80% of the length of a prediction interval). The magnitude of the difference in the interval estimates is fairly consistent across models with various sample sizes. In other words, we are concentrating for the data generated in the validation phase. In this case, the run-to-run variances and the within-run or repeatability variances are often different. Hence, the two intervals (i.e. β-expectation tolerance and prediction interval) are not equal.

On the other hand, in a recent article published by the same authors [9], a bioanalytical method dedicated to the determination of ketogluratic acid (KG) and hydroxymethylfurfural (HMF) in human plasma by SPE-HPLC-UV were validated using the frequentist risk profile, β-expectation tolerance interval and the reliability profile through the Bayesian probability.

Analysis of results was paradoxical. The statistical tools used to determine the validity of the method lead to different decisions. All the way through this article, one can clearly see that the β-expectation tolerance interval would define the analytical method as reliable over the whole concentration range tested, while the Bayesian one reduces the valid concentration range. This was explained by the fact that Bayesian methods provide accurate and more precise estimation of the reliability probability. Even more, when we applied the 95-content 95-confidence tolerance interval to assess the validity of the method for determining the ketogluratic acid, we found that the Bayesian reliability profile and the β-content, γ-confidence tolerance interval have led, approximately at the same decision (see Table 1).

Moreover, we have registered in [10] that the uncertainty estimated in the validation stage using the β-expectation tolerance interval is always less than the uncertainty assessed in routine phase. Again, the comparison of measurement uncertainty estimations of cidofovir determination method [10] at each concentration level investigated during the validation phase using β-expectation tolerance limits and β-content, γ-confidence tolerance interval, has shown that our strategy better approximates the measurement uncertainty of routine phase (see Table 2).

As well, Marini et al. [11] have noted a difference between the uncertainty evaluated by the β-expectation tolerance interval with those calculated by robustness and inter-laboratory study. As a consequence, we can state that this type of statistic (i.e. β-expectation tolerance interval) (i) cannot predict future measurements of the method in routine phase, since it is unable to correctly estimate the measurement uncertainty; and (ii) is unfortunately not able to protect simultaneously the laboratory and the client interests (favors the laboratory to the detriment of the client).

In order to complete this discussion, we would like to point out that we have compared the results obtained by the 66.7-content 90-confidence tolerance interval, with that calculated by 95-expectation tolerance each time leads to the same decision [2]. But, our preference is for the β-content, γ-confidence tolerance interval, because this type of interval allows a better estimate of measurement uncertainty compared to the β-expectation tolerance interval and it perfectly translates the 4-6-λ rule recommended by FDA [12–14]. Other statistical tools, however, can be recommended to evaluate the performance of analytical methods [15].

0039-9140/$ – see front matter 
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**Table 1**
Assessment of reliability of the method dedicated to the determination of KG in human plasma by using the following statistical tools: β-expectation tolerance limit, β-content, γ-confidence tolerance interval and Bayesian reliability profile (acceptance limits λ = ±20%).

| Statistical tool | Concentration level ($\mu g\,mL^{-1}$) | Tolerance limits (%) | Decision |
|---|---|---|---|
| 90-expectation tolerance limits | 0.1333 | [−17.25; 7.28] | Valid |
| | 0.6667 | [−5.25; 14.34] | Valid |
| | 13.33 | [−11.33; −0.45] | Valid |
| | 133.3 | [−0.60; 5.78] | Valid |
| 90-content, 95-confidence tolerance intervals | 0.1333 | [−42.19; 32.19] | Invalid |
| | 0.6667 | [−24.79; 34.80] | Invalid |
| | 13.33 | [−23.51; 11.52] | Invalid |
| | 133.3 | [−5.83; 11.03] | Valid |
| Bayesian reliability profile | 0.1333 | – | Invalid |
| | 0.6667 | – | Invalid |
| | 13.33 | – | Invalid |
| | 133.3 | – | Valid |

**Table 2**
measurement uncertainty estimations of cidofovir determination method at each concentration level investigated during validation phase (using 95-expectation tolerance limits (%) and 90-content, 95-confidence tolerance interval) and during routine stage. The expanded uncertainty was computed using a coverage factor of 2.

| Study name | Concentration level ($ng\,mL^{-1}$) | Relative expanded uncertainty (%) |
|---|---|---|
| Validation: 95-expectation tolerance limits(%) | 50 | 55.5 |
| | 100 | 16 |
| | 150 | 20.1 |
| | 500 | 7.1 |
| | 1020 | 10.1 |
| Validation: 90-content, 95-confidence tolerance interval | 50 | 90.6 |
| | 100 | 26.4 |
| | 150 | 29.0 |
| | 500 | 11.6 |
| | 1020 | 16.7 |
| Routine: Trial 1 | 150 | 26.9 |
| | 500 | 13.5 |
| | 850 | 13.0 |

At the end, the applicability of our approach for determining the validity of the bioanalytical methods using different instrumental technique will be published shortly.

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