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Response to Letter to the Editor

Reply to the responses on the comments on "Uncertainty profiles for the validation of analytical methods" by Saffaj and Ihssane

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ABSTRACT

Saffaj and Ihssane, recently proposed an uncertainty profile for evaluating the validity of analytical methods using the statistical methodology of γ-confidence β-content tolerance intervals. This profile assesses the validity of the method by comparing the method measurement uncertainty to a predefined acceptance limit stating the maximum uncertainty suitable for the method under study. In this letter we comment on the response (T. Saffaj, B. Ihssane, Talanta 94 (2012) 361–362) these authors have made to our previous letter (E. Rozet, E. Ziemons, R.D. Marini, B. Boulanger, Ph. Hubert, Talanta 88 (2012) 769–771). In particular, we demonstrate that β-expectation tolerance intervals are prediction intervals, we show that β-expectation tolerance intervals are highly useful for assessing analytical methods validation and for estimating measurement uncertainty and finally we show what are the differences and implications for these two topics (validation and uncertainty) when using either the methodology of β-expectation tolerance intervals or the γ-confidence β-content tolerance tolerance interval one.

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Since the publication of the manuscript entitled "Uncertainty profiles for the validation of analytical methods" by Saffaj and Ihssane [1] we have initiated a discussion [2] that has led to a reply by Saffaj and Ihssane [3]. This letter aims at clarifying points that have been raised by Saffaj and Ihssane in their reply [3].

1. β-Expectation tolerance intervals are prediction intervals

First the relationship between β-expectation tolerance intervals and prediction intervals needs to be further explained as in their recent document Saffaj and Ihssane argue that they are different [3].

The formal definition of a prediction interval $[L,U]$ is [4,5]:

$$\begin{aligned} & P(X_{n+1} \in [L,U]) \geq \beta \\ & \Updownarrow \\ & P(L(X_1,\ldots,X_n) \leq X_{n+1} \leq U(X_1,\ldots,X_n)) \geq \beta \\ & \Updownarrow \\ & P(X_{n+1} \leq U(X_1,\ldots,X_n)) - P(X_{n+1} \leq L(X_1,\ldots,X_n)) \geq \beta \\ & \Updownarrow \\ & E(F[U(X_1,\ldots,X_n)] - F[L(X_1,\ldots,X_n)]) \geq \beta \end{aligned} \quad (1)$$

where F is the cumulative density function (cdf) and let $X_1,X_i\ldots,X_n$ be independent and identically distributed (iid) with the cdf F.

The formal definition of a β-expectation tolerance interval $[L,U]$ is [6]:

$$\begin{aligned} & E(P[L \leq X_i \leq U]) \geq \beta \\ & \Updownarrow \\ & E[P(X_i \leq U) - P(X_i \leq L)] \geq \beta \\ & \Updownarrow \\ & E[F[U] - F[L]] \geq \beta \end{aligned} \quad (2)$$

From Eqs. (1) and (2) we can see that β-expectation tolerance intervals are prediction intervals for a single future value. This is already known from the literature of statistical intervals [7–11].

In addition, in their letter [3] Saffaj and Ihssane cite two documents [4,5] that they use to justify that prediction intervals and β-expectation tolerance intervals are different in the case of linear mixed models such as the random on way analysis of variance (ANOVA) model used in analytical method validation studies. However, when reading these two documents there is no such affirmation that prediction intervals and β-expectation intervals are different. They are identical concepts and we provide here examples of references that further justify our statement: see e.g. [7–11].

It is only the initial interpretation of prediction intervals and β-expectation tolerance intervals that differs. The former is interpreted as an interval where a future result has a probability β to fall in. The last one is an interval where on average, a proportion β of the population will fall in. However, these interpretations, exactly as the formal definitions of the respective intervals, are equivalent. If each result has a probability β to fall in an interval, then, on average, a proportion β of the population will fall in this interval [7–13].

2. The case of linear mixed models

Then, what is the problem when several variance components are present in linear mixed models such as in the specific case of analytical method validation [14] or transfer [15]?

The only core problem is that there is no exact statistical solution to obtain a prediction interval or a β-expectation interval (remember: they are equivalent). In these cases, the statistical solutions are only approximations. The formula that we use in our methodology is a β-expectation tolerance interval proposed by

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Mee [16] and is only an approximation. The formulas of the β-content, γ-confidence tolerance intervals used by Saffaj and Ihssane in their paper [1] are also only approximations. Even the computation of the degrees of freedom used following the Satterthwaite formula is an approximation [17]. Also, in the documents referenced by Saffaj and Ihssane used to justify the difference between β-expectation tolerance intervals and prediction intervals, the estimators used to obtained the prediction intervals (or equivalently the β-expectation tolerance intervals) are also approximations [4,5]. In one of this document the authors proposed up to three different estimators to obtain prediction intervals [4].

In our recent article using Bayesian statistics [18], there were also three different estimators used to compute the probability to obtain future analytical results within pre-specified acceptance limits. In another recent article Saffaj and Ihssane also used a Bayesian estimator of the β-expectation tolerance intervals [19]. Each estimator provides different length of the β-expectation tolerance intervals. While frequentist approaches rely on approximations, Bayesian approaches do not and should provide more precise tolerance intervals estimations. This is what has been showed for the case of the estimation of the probability of obtaining reliable results over a concentration range investigated in [18]. In addition, this last paper has not compared the Bayesian reliability estimation to the β-content, γ-confidence tolerance interval approach with the simulations performed. It is then difficult to objectively confirm that the Bayesian approach developed in [18] and the β-content, γ-confidence content tolerance interval approach provide identical intervals. In fact, they are not. In our document [18], we have computed the mean posterior predictive probability to compare it with the probability estimated by the β-expectation tolerance interval. Whereas β-content, γ-confidence will provide a lower bound to this estimated probability [11]. In addition to compute Bayesian β-content, γ-confidence tolerance interval another algorithm should have been used [20].

3. What tolerance interval for method validation?

The point of our first comment [2] was to stress the difference in interpretation of β-expectation tolerance intervals and β-content, γ-confidence tolerance intervals. With the former we showed that a good compromise was made between consumer and producer risk, with the latter, the consumer risk is also well controlled while the producer risk is, to our view, excessive [2,21]. Nonetheless, when practitioners will have to choose between one tolerance interval and the other, this risk evaluation should be made. Evidently, a balance between risks mitigation and time and costs will be required. Finally, it is important to stress that in comparison to any other classical decision methodology used in the context of method validation the use of tolerance intervals (β-expectation or β-content, γ-confidence) provides the best guarantees concerning the decision of declaring a method as valid. They both look at the reliability of the analytical results generated by the analytical method.

4. Method validation and measurement uncertainty

The question about what interval estimates the best the measurement uncertainty is also raised by Saffaj and Ihssane in their response [3]. Using β-content, γ-confidence tolerance intervals will always provide measurement uncertainty estimations larger than when using β-expectation tolerance intervals. In the former the uncertainty of the uncertainty is fully computed and integrated, while in the last one the uncertainty of the uncertainty is only included in the quantile Students-t distribution. Hence the formula used by Saffaj and Ihssane in their first article (second case of formula (31) in Ref. [1]), will always provide an estimation of measurement uncertainty larger than the one provided by β-expectation tolerance intervals. This directly comes from the way tolerance intervals are built: an upper bound to the variance components is searched for when building a β-content, γ-confidence tolerance interval (see e.g. [1,22]).

In the paper of Lecomte et al. [23], the uncertainty estimated from the method validation was found more or less close to the uncertainty of the two routine trials used, depending on the concentration level and on the routine trial. The estimation of uncertainty provided by Saffaj and Ihssane [3] has been shown to be relatively close to the uncertainty obtained in routine trial 1. However, when comparing to trial 2, the uncertainty estimated by the methodology proposed by Saffaj and Ihssane [3] is exceeding the one obtained from the routine runs.

In the paper of Marini et al. [24], the sources of uncertainty are not the same in the validation study, the robustness study and the inter-laboratories study. These last two studies include naturally more sources of uncertainty than an in-house method validation study. This explains the difference of uncertainty estimations. In addition, the true measurement uncertainty is not known, so it is difficult to decide which approach is providing the best estimate. The key message of this article was to show that robustness studies provided measurement uncertainty estimates that were close to uncertainty estimates obtained from inter-laboratories. Another message was also to show that measurement uncertainty for a single laboratory obtained from robustness studies, and inter-laboratories studies were providing estimation of measurement uncertainty that were exceeding those obtained from an in-house method validation.

None of the approaches using either β-expectation tolerance intervals or β-content, γ-confidence tolerance intervals will provide perfect estimates of the "routine uncertainty". In some instances the β-expectation tolerance interval approach for measurement uncertainty will provide smaller estimation than the "routine uncertainty" and in others closer to it. Similarly, the estimates provided by the β-content, γ-confidence tolerance intervals will in some cases provided higher estimations than the "routine uncertainty" and in other cases closer ones to it. This behavior will highly depend on the experimental design used in the method validation that should include the most important sources of uncertainty. Both of these estimators of measurement uncertainty are only initial estimates of the measurement uncertainty and should be updated during the daily use of the method. Quality control samples used during the routine application of the method could valuably be used to achieve this. In addition, including other sources of uncertainty either type A or type B could also be performed. And, if Bayesian approach is used such as the one proposed by Saffaj and Ihssane [19], adequate informative priors could also help in increasing the precision of the estimators of measurement uncertainty.

In conclusion we would like to stress that, β-expectation tolerance intervals:

i. Are perfectly able to predict future routine results in coherence with their statistical definition. This has been shown in numerous occasions, see Refs. [23,25].
ii. Are able to provide an initial estimation of the routine measurement uncertainty, the reliability of which depends on the amount of data and on the experimental setup [26–28]. This dependency is equally valid for the case using β-content, γ-confidence tolerance intervals.
iii. Are providing an adequate balance between consumer risk and producer risk [2,21,27].

The selection of one tolerance interval or the other is at the hand of the analysts or of the regulatory bodies. It will be a

balance between risk analysis and cost analysis. As summarized in this letter, we favor the use of β-expectation tolerance intervals for assessing the validity or transferability of analytical methods and the estimation of measurement uncertainty. We rather recommend the use of β-content, γ-confidence tolerance intervals to evaluate whether a proportion e.g. of a batch of products, will be in predefined specification limits as used e.g. in the pharmacopoeias for the evaluation of Uniformity of Dosage Units [29]. Nonetheless, both of these approaches (and others) are going the right way by assessing the reliability of analytical results and not only providing a diagnostic of the performances of the analytical procedure itself.

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References

[1] T. Saffaj, B. Ihssane, Talanta 85 (2011) 1535–1542.
[2] E. Rozet, E. Ziemons, R.D. Marini, B. Boulanger, Ph. Hubert, Talanta 88 (2012) 769–771.
[3] T. Saffaj, B. Ihssane, Talanta 94 (2012) 361–362.
[4] T.Y. Lin, C.T. Liao, J. Stat. Plann. Inference 138 (2008) 3164–3175.
[5] C.M. Wang, Commun. Stat. Simul. Comput. 21 (1992) 671–687.
[6] I. Guttman, Statistical Tolerance Regions: Classical and Bayesian, Hafner, Darian, 1969 147 p.
[7] R.W. Mee, Technometrics 32 (1990) 83–92.
[8] G.J. Hahn, J. Am. Stat. Assoc. 64 (1969) 878–888.
[9] R.E. Odeh, Technometrics 32 (1990) 203–216.
[10] T.Y. Lin, C.T. Liao, Comput. Stat. Data Anal. 50 (2006) 911–925.
[11] R.W. Mee, Technometrics 31 (1989) 99–105.
[12] G.J. Hahn, W.Q. Meeker, Statistical Intervals: A Guide for Practitioners, Wiley Edition, 1991 392 p.
[13] S.B. Vardeman, Am. Stat. 46 (1992) 193–196.
[14] A. Bouabidi, E. Rozet, M. Fillet, E. Ziemons, E. Chapuzet, B. Mertens, R. Klinkenberg, A Ceccato, M. Talbi, B. Streel, A. Bouklouze, B. Boulanger, Ph. Hubert, J. Chromatogr. A 1217 (2010) 3180–3192.
[15] W. Dewé, B. Govaerts, B. Boulanger, E. Rozet, P. Chiap, Ph. Hubert, Chemom. Intell. Lab. Syst. 85 (2007) 262–268.
[16] R.W. Mee, Technometrics 26 (1984) 251–254.
[17] F. Satterthwaite, Psychometrika 6 (1941) 309–316.
[18] E. Rozet, B. Govaerts, P. Lebrun, K. Michail, E. Ziemons, R. Wintersteiger, S. Rudaz, B. Boulanger, Ph. Hubert, Anal. Chim. Acta 705 (2011) 193–206.
[19] T. Saffaj, B. Ihssane, Talanta 92 (2012) 15–25.
[20] M. Hamada, V. Johnson, L.M. Moore, J. Wendelberger, Technometrics 46 (2004) 452–459.
[21] B. Boulanger, E. Rozet, F. Moonen, S. Rudaz, Ph. Hubert, J. Chromatogr. B 877 (2009) 2235–2243.
[22] D. Hoffman, R. Kringle, J. Biopharm. Stat. 15 (2005) 283–293.
[23] F. Lecomte, C. Hubert, S. Demarche, C. De Bleye, A. Dispas, M. Jost, F. Frankenne, A. Ceccato, E. Rozet, Ph. Hubert, J. Pharm. Biomed. Anal. 57 (2012) 153–165.
[24] R.D. Marini, P. Chiap, B. Boulanger, S. Rudaz, E. Rozet, J. Crommen, Ph. Hubert, Talanta 68 (2006) 1166–1175.
[25] E. Rozet, C. Hubert, A. Ceccato, W. Dewé, E. Ziemons, F. Moonen, K. Michail, R. Wintersteiger, B. Streel, B. Boulanger, Ph. Hubert, J. Chromatogr. A 1158 (2007) 126–137.
[26] M. Feinberg, B. Boulanger, W. Dewe, Ph. Hubert, Anal. Bioanal. Chem. 380 (2004) 502–514.
[27] E. Rozet, S. Rudaz, R.D. Marini, E. Ziemons, B. Boulanger, Ph. Hubert, Anal. Chim. Acta 702 (2011) 160–171.
[28] E. Rozet, R.D. Marini, E. Ziémons, W. Dewé, S. Rudaz, B. Boulanger, Trends Anal. Chem. 30 (2011) 797–806.
[29] European Pharmacopoeia (Ph. Eur.) 7th edition. Uniformity of Dosage Units, General Chapter 2.9.40.

E. Rozet *[1], E. Ziemons, R.D. Marini, Ph. Hubert
Laboratory of Analytical Chemistry, CIRM, Institute of Pharmacy, University of Liège, CHU, B36, Tour 4, Avenue de l'Hôpital 1, B-4000 Liège, Belgium
E-mail address: eric.rozet@ulg.ac.be (E. Rozet)

B. Boulanger
Arlenda SA, Avenue de l'Hôpital 1, B-4000 Liège, Belgium

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* Corresponding author. Tel.: +32 4366 4320; fax: +32 4366 4317.
[1] F.R.S.–FNRS Post-Doctoral Researcher (Belgium).