Talanta 106 (2013) 155–157

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

ARTICLE INFO

Keywords:
Method validation
Measurement uncertainty
β-Expectation tolerance interval
β-Content tolerance interval

ABSTRACT

This letter provides an opportunity to expand further on the problems associated with using tolerance interval for share validated analytical methods and to estimate the measurement uncertainty. Indeed, we propose at clarifying points that have been raised by Rozet et al. (Talanta 100 (2012) 290–292) as some of them merit to be discussed.

In that respect, we demonstrate here that β-content, γ-confidence tolerance intervals will provide perfect estimates of the routine uncertainty. In particular, we confirm that there is "no statistically significant difference" between the uncertainties estimated by our methodology with those obtained from the routine phase. Obviously, this is an opportunity to show which of the two types of tolerance interval i.e., β-expectation tolerance interval or β-content, γ-confidence tolerance interval allows a perfect estimate of the routine uncertainty.

Furthermore, we prove that the β-expectation tolerance interval does not provide an adequate balance between consumer risk and producer risk.

1. Introduction

We welcome the reply by Rozet et al. [1] on our responses [2] to the comments [3] on some aspects of our previous work entitled "Uncertainty profiles for the validation of analytical methods" [4] as it provides an opportunity to expand further on the problems associated with using tolerance interval for share validated analytical methods and to estimate the measurement uncertainty. This letter aims at clarifying points that have been raised by these authors as some of them merit to be discussed.

Firstly, we would like to start discussion, with the last point treated by Rozet et al. [1] in their reply i.e., the measurement uncertainty. It is important to note that the measurement uncertainty is a statistical parameter which describes the possible fluctuations of the analytical result. The uncertainty expanded by a factor 2 for e.g., is interpreted as an interval in which the true value of the result of a measurement resides with a defined probability. For this reason, this parameter is estimated in order to judge the adequacy of a result for its intended purpose and to verify its reliability with other comparable results. All together, measurement uncertainty with method validation are able to provide a way to ensure whether an analytical method is suitably fit for the purpose of meeting official requirements. So the positive sense of "measurement uncertainty" should be communicated to the superiors of the laboratory and to the consumers.

We return to the example of Lecomte et al. [5] since this article allows a comparison between the estimated uncertainty as of validation and from the routine trials. In their comments, Rozet et al. [1] concluded with this sentence "In the paper of Lecomte et al. [5], 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 [4] has been shown to be relatively close to the uncertainty obtained in routine trial1. However, when comparing to trial 2, the uncertainty estimated by the methodology proposed by Saffaj and Ihssane [4] is exceeding the one obtained from the routine runs". We demonstrate here that β-content, γ-confidence tolerance intervals will provide perfect estimates of the routine uncertainty. In particular, we show that there is "no statistically significant difference" between the uncertainties estimated by our methodology with those obtained from routine phases (trial 1 and trial 2).

In that respect, we would like to take advantage of the data provided by Lecomte et al. [5] and apply our procedure to the same cluster to either validate our approach or understand its limitations and pitfalls [4]. Obviously, this is an opportunity to show which of the two types of tolerance interval i.e., β-expectation tolerance interval or β-content, γ-confidence tolerance interval allows a perfect estimate of the routine uncertainty.

In order to compare the estimations of measurement uncertainty obtained from the method validation (β-content tolerance interval) versus those obtained from routine applications of a chromatographic method for the determination of cidofovir, we tested the following model:

$$u_c = \beta_0 + \beta_1 C + \beta_2 study + \beta_{12} study{*}C + \beta_{11} C^2 + \varepsilon \quad (1)$$

Where u_c is the uncertainty, C the cidofovir concentration in ng/mL and ε, the residual error is assumed normally distributed $N(0,\sigma^2)$. The coefficient of determination R^2 of this model equals to 0.97 signifying that the uncertainty is adequately modeled by this polynomial regression. After the validation of the model, we proceeded to statistically compare the various uncertainty estimates obtained in the various study sets. Indeed, various hypotheses have been tested by using contrast t-test. The estimated contrasts presented in Table 1 show clearly that $u_{validation}$, $u_{routine1}$

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.12.002

and $u_{routine2}$ uncertainty estimates are not significantly different. This result is evident when looking at the graphic of Fig. 1. We can therefore declare that the β-content, γ-confidence tolerance interval is able to predict perfectly the routine uncertainty. Another important point that can be concluded from this comparison that the experimental design adopted during the validation of this chromatographic method is largely sufficient to assess the uncertainty in phase routine.

In addition, for having the clearest image we proceeded to redo the comparison between the estimated uncertainties of the different phases, but this time by using the β-expectation tolerance interval instead of the β-content, γ-confidence tolerance interval. We found the coefficient of determination R^2 of this model equals to 0.98 suggesting that the uncertainty is also effectively modeled by the Eq. (1). Then, the contrast t-test has been used to statistically compare the various uncertainty estimates. The results of the test were collected in Table 2. Contrary to the first analysis, the estimated contrasts presented in Table 2 show clearly that uncertainty estimated from the method validation is significantly smaller than the uncertainty of the routine trial 1 (Fig. 2). This result leads us to believe that the β-expectation tolerance interval is highly dependent on the experimental design used in the method validation. This dependency is not equally valid for the case using β-content, γ-confidence tolerance interval. Indeed, the β-expectation tolerance interval needs other additional experiments to provide a perfect estimate of the uncertainty. For example, the method of Gonzalez et al. [7] may be recommended, which consists to add uncertainty evaluated by β-expectation tolerance interval with that calculated in robustness phase for a good estimate of the uncertainty. Thus our criticisms of SFSTP approach are reinforced by the contrasts presented in Tables 1 and 2 and clearly indicate the superiority of our approach to estimate the measurement uncertainty. The one thing we should know that in many cases the measurement uncertainty is more important than the measure itself. The question which now arises:"if the β-expectation tolerance interval is unable to predict the routine uncertainty how is it going to be able to predict future routine results"?

Related to this debate, we additionally show that this type of statistic (i.e., β-expectation tolerance interval) does not provide an adequate balance between consumer risk and producer risk. Due to the following reasons:

(i) It cannot predict future measurements of the method in routine phase since it is incapable to rightly assess the routine uncertainty.

(ii) We welcome the data provided by Rozet et al. [6] as it clearly vindicate our view that β-expectation tolerance interval is unfortunately not able to protect simultaneously the laboratory and the client interests (favors the laboratory to the detriment of the client). The results gathered in Table 3 confirms this finding since the Bayesian reliability profile and the SFSTP approach lead to different decisions a propos the validity of the bioanalytical method dedicated to the determination of ketogluratic acid and hydroxymethylfurfural in human plasma by SPE-HPLC-UV. Rozet et al. [6] have commented on these inconsistencies by the fact that Bayesian methods provide accurate and more precise estimation of the reliability probability. Indeed, this example is an irrefutable proof that the β-expectation tolerance interval excessively protects the producer risk to the detriment of the consumer risk.

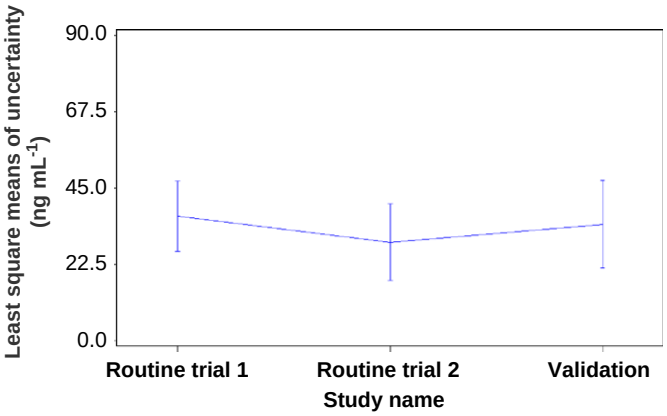

Fig. 1. Least square means plot of uncertainty as function of validation (β-content, γ-confidence tolerance interval), routine trial 1 and routine trial 2 studies.

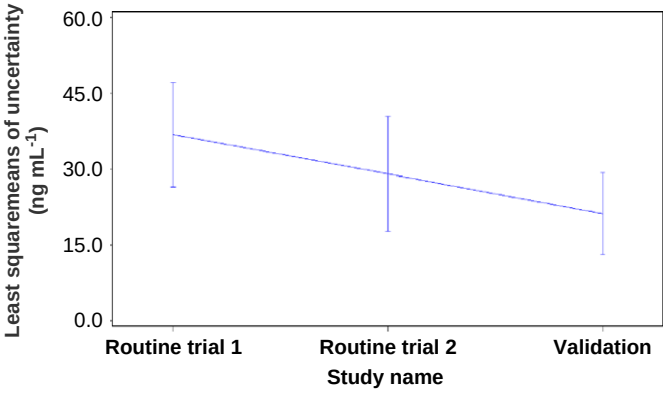

Fig. 2. Least square means plot of uncertainty as function of validation (β-expectation tolerance interval), routine trial 1 and routine trial 2 studies.

Table 1
Comparison of measurement uncertainty obtained during the method validation (β-content, γ-confidence tolerance interval) with the one obtained during the routine use of the chromatographic method for the determination of cidofovir.

	Contrast by *t*-test	
	Comparison $u_{validation}$ vs. $u_{routine\ trial\ 1}$	**Comparison $u_{validation}$ vs. $u_{routine\ trial\ 2}$**
Estimated difference	1.092	9.132
S.E.	5.008	5.008
***t*-Ratio**[a]	0.218	1.823
***p*-Value**	0.8381	0.1423

[a] $t_{table} = 2.776$.

Table 2
Comparison of measurement uncertainty obtained during the method validation (β-expectation tolerance interval) with the one obtained during the routine use of the chromatographic method for the determination of cidofovir.

	Contrast by *t*-test	
	Comparison $u_{validation}$ vs. $u_{routine\ trial\ 1}$	**Comparison $u_{validation}$ vs. $u_{routine\ trial\ 2}$**
Estimated difference	−12.020	−3.980
S.E.	3.373	3.373
***t*-Ratio**[a]	−3.564	−1.180
***p*-Value**	0.0235	0.3034

[a] $t_{table} = 2.776$.

Table 3
Assessment of reliability of the method dedicated to the determination of KG and HMF in human plasma by using the following statistical tools: β-expectation tolerance limit and Bayesian reliability profile. (Acceptance limits $\lambda = \pm 20\%$)

Statistical tool	Concentration level ($\mu g\ mL^{-1}$)	Tolerance limits (%) (KG/HMF)	Decision (KG/HMF)
90-expectation tolerance limits	0.1333	[−17.25;7.28]/[−14.10;8.25]	Valid/Valid
	0.6667	[−5.25;14.34]/[−4.44;3.39]	Valid/Valid
	13.33	[−11.33;−0.45]/[1.05;7.05]	Valid/Valid
	133.3	[−0.60;5.78]/[−6.83;10.35]	Valid/Valid
Bayesian reliability profile	0.1333	–	Invalid/Invalid
	0.6667	–	Invalid/Invalid
	13.33	–	Invalid/Invalid
	133.3	–	Valid/Valid

To conclude this letter, we wish to emphasize that we have identified other weaknesses in the use of the β-expectation tolerance interval to evaluate the performance of analytical methods, which deserves to be developed in another separate article. Nevertheless, we must recognize that in comparison to any other classical decision methodology used in the context of method validation the use of tolerance intervals (β-expectation tolerance interval or β-content, γ-confidence tolerance interval) provides the best guarantees concerning the decision of declaring a method as valid. For this reason, we believe that these two types of tolerance intervals complement each other and if we take into account the strengths of each interval can lead to an overall strategy for the analytical validation and the estimation of measurement uncertainty. In the meantime, the Bayesian β-expectation tolerance interval is strongly recommended to perform this role [8].

References

[1] E. Rozet, E. Ziemons, R.D. Marini, B. Boulanger, Ph. Hubert, Talanta 100 (2012) 290–292.
[2] T. Saffaj, B. Ihssane, Talanta 94 (2012) 361–362.
[3] E. Rozet, E. Ziemons, R.D. Marini, B. Boulanger, Ph. Hubert, Talanta 88 (2012) 769–771.
[4] T. Saffaj, B. Ihssane, Talanta 85 (2011) 1535–1542.
[5] 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.
[6] 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.
[7] A.G. Gonzalez, M.A. Herrador, Talanta 70 (2006) 896–901.
[8] T. Saffaj, B. Ihssane, Talanta 92 (2012) 15–25.

T. Saffaj*, B. Ihssane
Centre Universitaire Régional d'Interface Université Sidi Mohamed Ben Abdallah, BP 2626, route d'Imouzzar-Fès, Morocco

Laboratoire de Chimie Organique Appliquée Faculté des Sciences et Techniques, BP 2202, route d'Imouzzar-Fès, Morocco
E-mail address: saffajt@gmail.com (T. Saffaj)

Received 25 November 2012
2 December 2012
3 December 2012

Available online 21 December 2012

* Corresponding author at: Université Sidi Mohamed Ben Abdallah, Chemistry, Centre Universitaire Régional d'Interface, route d'Imouzzar-Fès, BP 2626, Morocco.