Evaluating analytical results reliability using a Bayesian probability criterion

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1 Introduction

Methods validation is mandatory in order to assess the fitness of purpose of developed analytical method such as HPLC, GC, etc. Of core importance at the end of the validation is the evaluation of the reliability of the individual results that will be generated during the routine application of the method. Regulatory guidelines provide a general framework to assess the validity of a method, but none address the issue of results reliability. In this study, a proposed Bayesian approach provides the effective probability of obtaining reliable future analytical results over the whole concentration range investigated. This is summarized in a single graph: the reliability profile. This Bayesian reliability profile is also compared to a frequentist approach derived from the work of Dewé et al. [1]. Furthermore, to illustrate the applicability of the Bayesian reliability profile, this approach is applied to a bioanalytical method dedicated to the determination of ketoglutaric acid (KG) and hydroxymethylfurfural (HMF) in human plasma by SPE-HPLC-UV..

2 Material and methods

2.1 Bayesian model

Results of analytical method (*k* repetitions, *j* runs, *i* concentration levels) are assumed providing from a normal distribution: $X_{ijk} \sim N\left(\Delta_{i,j}\ ;\ \sigma_i\right)$ Eq. 1

The mean of this distribution can be interpreted as the method bias function dependent on the true *ith* concentrations $\mu T,i$. The form chosen is thus a linear regression with random slopes and intercepts:

$$\Delta_{ij} = \alpha_j \mu_{T,i} + \beta_j \qquad \text{Eq. 2}$$

The regression coefficients for the *jth* run $\boldsymbol{\beta}_j = \left(\alpha_j, \beta_j\right)'$ are assumed coming from the model:

$$\boldsymbol{\beta}_j \sim N(\boldsymbol{\theta}, \sigma_\beta^2 \boldsymbol{\Sigma}) \qquad \text{Eq. 3}$$

Finally the following vague priors are defined:

$$\boldsymbol{\theta} \sim N(0, \boldsymbol{\Gamma})$$

$$\boldsymbol{\Gamma}^{-1} = \mathbf{0}$$

$$\boldsymbol{\Sigma} \sim \text{Wishart}(0.0001\mathbf{I}_2, 2)$$

where $\mathbf{I}_2$ represents the 2 x 2 identity matrix and $\boldsymbol{\Gamma}^{-1} = \mathbf{0}$ denotes a matrix of 0s that represents a vague prior of $\boldsymbol{\theta}$.

The standard deviation of the results is given as being dependent on the true concentration level: $\sigma_i = (\mu_{T,i})^{\gamma}$ Eq. 4

The vague prior used is: $\gamma \sim N(0,0.0001)$

Finally, having specified the regulatory or client specifications or acceptance limits (λ), the main aim is to obtain the reliability probability (P_{Rel}) as a function of the true concentration:

$$P_{\text{Rel}} = P\left[\left(\left|x_{ijk} - \mu_{T,i}\right| = \Delta_{ij} + \sigma_i\right) \leq \lambda \middle| \alpha_j, \beta_j, \gamma, \forall \mu_{T,i}\right] \geq P_{\min} \quad \text{Eq. 5}$$

MCMC sampling is then performed (for example, using R2Winbugs package from R), which allows us to obtain the posterior distribution of each parameter. The predictive distribution of the reliability probability for any true concentration level is then obtained following the next algorithm.

2.2 Reliability profile algorithm

For each concentration level covering the range that is being assessed:

1. Draw 10,000 regression parameters $\boldsymbol{\beta}_j^*$ from the posterior distribution of $\boldsymbol{\beta}_j$ (Eq. 3) as well as 10,000 coefficients of the precision function γ^* from Eq. 4.
2. For each of the 10,000 previously selected combination of bias and precision functions, draw 10,000 results X_{ijk}^* from Eq. 1, which represent a sample of the predictive distribution of the results.
3. Then compute the number of results lying inside the specifications or acceptance limits $[-\lambda\,;\,\lambda]$ for each of the 10,000 situations.
4. The posterior reliability probability $P_{\text{Rel}} = P\left(X \middle| \Delta_{ij}, \sigma_i, \gamma, data\right)$ at each concentration level is finally estimated by the proportion of results out of 10,000 included within the specification limits

Finally, a graph representing the reliability of the results obtained by a quantitative analytical method over the whole concentration range studied can be obtained, and the concentration range over which the method is sufficiently reliable can be determined by comparing the posterior reliability probability (P_{Rel}) to a minimum reliability value (P_{min}), for e.g. 95%.

2.3 Experimental

In order to illustrate the proposed approach, it was applied in the present study to the validation of an HPLC-UV assay for the simultaneous quantification of ketoglutaric acid (KG) and hydroxymethylfurfural (HMF) in human plasma. The validation design is:

- Calibration standards: 5 concentration levels, 2 repetitions per level, 3 runs.
- Validation standards: 4 concentration levels, 4 repetitions per level, 3 runs.

The specification (λ) = +/-20% around the true concentration values of the validation standards. The minimum reliability criterion (P_{min}) was set at 90%

3 Results and discussions

The whole Bayesian procedure described here is applied to the HPLC-UV assay and the reliability profiles can be worked out, as illustrated in Figure 1a and 1b for both analytes, respectively.

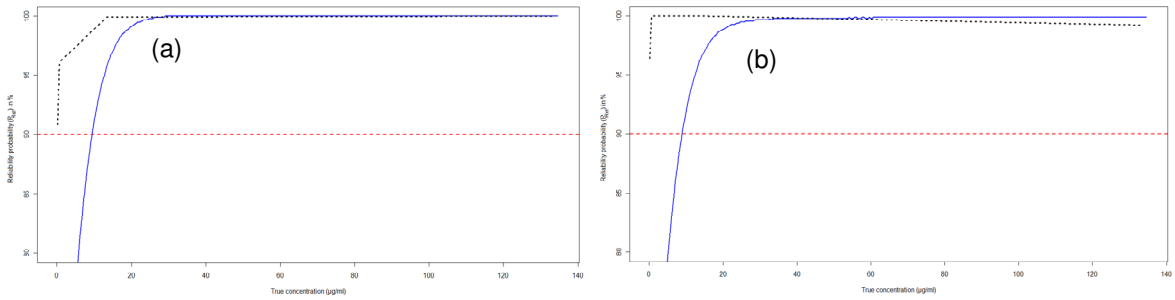

Figure 1 – Reliability profile for (a) KG and (b) HMF depicting on the y-axis the reliability probability (P_{Rel}, in %) i.e. the probability to obtain future analytical results within pre-specified acceptance limits λ=±20% with respect to the true concentration of analytes on the x-axis. Continuous line: Bayesian reliability profile; Dotted line: frequentist reliability profile. Dashed horizontal line: minimum reliability probability (P_{min} in %) set at 90%.

In these profiles, the minimum reliability criterion (P_{min}) of 90% is shown by the dashed horizontal lines. The concentration levels with at least 90% reliability define the valid concentration range for KG and HMF and consequently represent the lower and upper quantification limits. The valid concentration range using the Bayesian algorithm is from 9.4 to 133.3 µg/ml and 8.9 to 133.3 µg/ml for KG and HMF respectively. In Figure 1a and 1b, the frequentist reliability profiles are also drawn in order to compare the two approaches. As expected, the Bayesian risk and reliability profiles are more conservative. Indeed, this approach not only models the probabilities over the whole concentration range studied, but it also includes the uncertainty of each parameter involved in the bias and imprecision function and thus appears more strict when compared to the frequentist approach. However, one advantage of the Bayesian reliability profile is the possibility of knowing the quality of the results of a quantitative analytical method obtained at all concentration levels rather than merely at those levels studied in the validation phase. A second advantage of these profiles is that the inclusion of the uncertainty of the parameters should ultimately make the decision of validity more trustworthy and should lead to an increase in reliability for final users of the results such as clients, patients and so on.

4 Conclusions

Quantitative analytical methods play a central role as the generated analytical results are used to make highly critical decisions such as conformity of products with release or legal specifications. Analytical results might also impact the health of a patient or cause the premature ending of preclinical or clinical studies. In this study, a novel Bayesian proposition was made in order to evaluate the reliability of analytical methods over a defined concentration range. This leads to a reliability profile over which the lower and upper quantification limits are appropriately located and thus the analytical method valid range can be obtained by comparison with a minimum reliability probability. The comparison of this approach with a frequentist one showed that the Bayesian approach was more conservative, a fact that is not inappropriate considering the highly important health and financial decisions that will be made using the validated methods in routine applications. Finally, the Bayesian approach is in full agreement with the regulatory validation guidelines since all the required validation criteria are included.

5 References

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