A Bayesian Predictive Probability Criterion to Assess Analytical Methods Validity

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INTRODUCTION & AIM

- 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. (*Dewé W., Govaerts B., Boulanger B., Rozet E., Chiap P., Hubert Ph., Chemometr. Intell. Lab. Syst. 2007, 85, 262-268*).
- 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.

BAYESIAN MODEL

Results of analytical method (*k* repetitions, *j* runs, *i* concentration levels) are assumed providing from a normal distribution:

$$X_{ijk} \sim N\left(\Delta_{i,j}\ ;\ \sigma_i\right) \qquad \text{Eq. 1}$$

The mean of this distribution can be interpreted as the method bias function dependent on the true i^{th} 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 j^{th} run $\boldsymbol{\beta}_j=(\alpha_j, \beta_j)'$ 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:

$$\log(\sigma_i) = \log(\sigma) + \gamma \log(\mu_{T,i}) \qquad \text{Eq. 4}$$

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 :

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

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.

RELIABILITY PROFILE ALGORITHM

From these posterior distributions one can then easily obtain the predictive distribution of the reliability probability P_{Rel} for any concentration level $\mu_{T,i}$. Indeed, from the posterior distribution of the parameters obtained with the MCMC sampling, the posterior reliability probability P_{Rel} of results following the normal distribution defined in Eq. 1 lying inside the acceptance limits λ is easily obtained analytically.

Finally, a graph representing the reliability of the results 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}), e.g. 95%.

EXAMPLE OF APPLICATION

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.

Results obtained with the proposed Bayesian approach are compared to the frequentist approximation derived from the previously described work of Dewé et al.

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%

Having set these requirements, the reliability profile can be worked out, as illustrated in Figures 1 and 2 for both analytes, respectively.

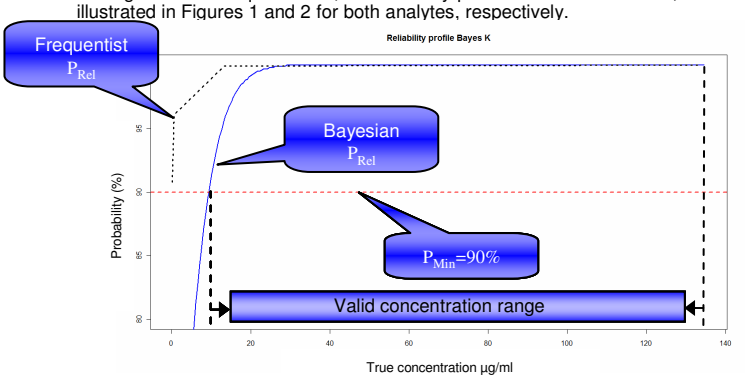

Fig. 1. Reliability profile for KG determination.

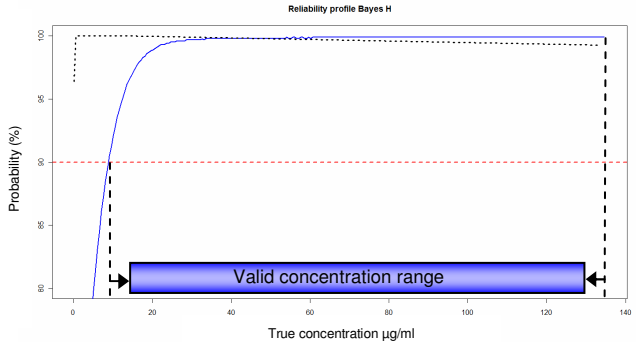

Fig. 2. Reliability profile for HMF determination.

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.

Finally, the Bayesian approach is in full agreement with the regulatory validation guidelines since all the required validation criteria are included.

ACKNOWLEDGMENTS

A research grant from the Belgium National Fund for Scientific Research (FRS-FNRS) to E. Rozet is gratefully acknowledged.

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RDPA 2011
Recent Developments in Pharmaceutical Analysis
14th International Meeting
September 21-24, 2011 - University of Pavia, Italy