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A Bayesian approach for application to method validation and measurement uncertainty

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ABSTRACT

The aim of this paper is to apply a new technique for the validation of quantitative analytical procedures based on Bayesian simulation and accuracy profile. Also, an original strategy for estimating measurement uncertainty by the same approach has been developed. The performance of our proposal was confirmed by application to analytical and bio-analytical methods. Compared to the classical strategy, the new approach has a more holistic character. It means that it is no longer necessary to know the various individual steps into which the analytical method can be broken down since this latter is taken as a whole.

Furthermore, the Bayesian accuracy profile procedure allows to control the risk associated with the future use of the analytical method.

1. Introduction

Analytical validation is a topic that has attracted many researchers in different fields of analytical chemistry. However, the question of acceptability of a quantitative analytical procedure remains unevenly and incompletely resolved. Indeed, several strategies by different standards and guides (ISO, ICH, FDA, AOAC, EURACHEM etc.) [1–10] have been proposed to carry out the analytical validation; the latest is based on the accuracy profiles estimated by tolerance interval [1].

Statistical tolerance intervals are useful in validation of analytical procedures, life-testing, process reliability studies, pharmaceutical engineering, and many other areas. Three basic types of tolerance intervals have received considerable attention: (i) β-content tolerance intervals, (ii) β-expectation tolerance intervals and (iii) fixed-in-advance tolerance intervals. The problem of constructing tolerance limits in the balanced one way random-effects model, and simple random sample (SRS) has been investigated by several authors.

For univariate normal distribution, tolerance intervals have been first discussed by Wilks [11] and Paulson [12]. Further, Fraser and Guttman [13] defined and constructed "good" tolerance intervals. Mee considers the β-expectation tolerance interval for balanced one-way random effects models and proposes an analytical approximate method based on the result of Wilks [14,15]. Indeed, the Mee's method has been adopted by the Société Francaise des Sciences et Techniques Pharmaceutiques (SFSTP2003) for the validation of quantitative analytical procedure [1,2].

Recently, Lin and Liao developed a β-expectation tolerance interval procedure for all mixed linear models [17]. Their method is based on the concept of generalized pivotal quantity, presented in Weerahandi [18]. We can define the β-expectation tolerance interval as an interval covers on average 100β% of the distribution given the estimated parameters. This interval is referred to by Mee [14] as a "mean-coverage" tolerance interval.

The derivation of tolerance interval in a Bayesian framework is first introduced by Aitchison [23,24], and the results are also given in Guttman [19]. Wolfinger has presented a simulation-based approach for determining Bayesian tolerance intervals in a balanced one-way random effects model [20]. The theory and results of Wolfinger have been extended to the balanced two-factor nested random effects model in Van der Merwe [21]. Also, a simulation approach for constructing Bayesian tolerance intervals in an unbalanced one-way random effects model are treated by the same author [22].

This article aims to exploit the Bayesian simulation given by Wolfinger for share validated analytical methods and to estimate the measurement uncertainty.

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At first, a reminder and mathematical definitions are presented for the construction of Bayesian tolerance interval. Secondly, the applicability of the proposed tolerance intervals is demonstrated in Section 3 by application to the problem of quantitative analytical method validation based on the accuracy profile and evidence of the interest of the method is provided by illustrative examples.

2. Chemometrical development

2.1. Bayesian tolerance intervals

2.1.1. One-way random effects model

During pre-study method validation, measurements are made over multiple independent assay runs with replicate determinations within each run. A statistical model to describe the measured values is given by [26]:

$$Y_{ij} = \mu + b_i + e_{ij}; \quad j = 1, 2, \ldots, n; \quad i = 1, 2, \ldots, a \tag{1}$$

Where Y_{ij} denotes the jth replicate observation corresponding to ith run, μ is an unknown general mean, b_i's represents random effects and e_{ij}'s represent error terms. It is assumed that b_i's and e_{ij}'s are all independent having the distributions $b_i \sim N(0, \sigma_b^2)$ $\tau_i \sim N(0, \sigma_\tau^2)$ and $e_{ij} \sim N(0, \sigma_e^2)$. Thus, $Y_{ij} \sim N(0, \sigma_\tau^2 + \sigma_e^2)$ and $\sigma_b^2 \sigma_\tau^2$ and σ_e^2 represent the two variance components in the model.

We define

$$\bar{Y} = \frac{1}{an}\sum_{i=1}^{a}\sum_{j=1}^{n} Y_{ij},\ \bar{Y}_i = \frac{1}{n}\sum_{j=1}^{n} Y_{ij},\ SS_b = n\sum_{i=1}^{a}(\bar{Y}_i - \bar{Y})^2 \text{ and } SS_e$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_i)^2 \tag{2}$$

Table 1 gives the analysis of variance (ANOVA) and the expected mean squares under the model (1).

2.1.2. Bayesian β-expectation tolerance interval

In order to introduce the key idea about Bayesian β-expectation tolerance interval, let X denotes a random variable whose distribution depends on a parameter θ, and the realization of $\mathbf{X}$ give the observed data. Here $\boldsymbol{\theta}$ could be a vector. Let $\mathbf{x}$ be a vector representing realizations of X, and let $L(x|\boldsymbol{\theta})$ be the likelihood function. If $\pi(\theta)$ denotes a prior distribution for $\boldsymbol{\theta}$, then the posterior distribution of θ, say $P(\boldsymbol{\theta}|x)$, is given by

$$P(\boldsymbol{\theta}|x) = \frac{L(x|\boldsymbol{\theta})\pi(\boldsymbol{\theta})}{\int L(x|\boldsymbol{\theta})\pi(\boldsymbol{\theta})d\theta} \tag{3}$$

A Bayesian tolerance interval for the univariate normal distribution has been originally derived by Aitchison [23,24], and the results are also given in Guttman [19].

Let's now consider the derivation of tolerance intervals for one-way random model with balanced data. Let $\bar{Y}$, SS_b and SS_e are independently distributed with

$$\bar{Y} \sim N\left(\mu, \frac{n\sigma_b^2 + \sigma_e^2}{an}\right),\ \frac{SS_b}{n\sigma_b^2 + \sigma_e^2} \sim \chi^2_{a-1} \text{ and } \frac{SS_e}{\sigma_e^2} \sim \chi^2_{a(n-1)} \tag{4}$$

The likelihood is taken to be joint density of the above three random variables, to be denoted by $L(\bar{y}, ss_b, ss_e|\mu, \sigma_b^2, \sigma_e^2)$, where $\bar{y}$, ss_b and ss_e denote the observed values of $\bar{Y}$ and SS_e respectively.

Let's now consider the derivation of Bayesian β-expectation tolerance intervals under the non-informative prior (5) for $(\mu, \sigma_b^2, \sigma_e^2)$.

$$\pi(\mu, \sigma_b^2, \sigma_e^2) \propto \frac{1}{\sigma_e^2(n\sigma_b^2 + \sigma_e^2)} \tag{5}$$

If we define

$$\Omega_b = n\sigma_b^2 + \sigma_e^2 \quad \text{and} \quad \Omega_e = \sigma_e^2 \tag{6}$$

Then the marginal posterior distributions of Ω_b and Ω_e are proportional to an inverse gamma distribution and can be easily worked out if we ignore the restricted parameter space $\Omega_b \geq \Omega_e$. These posterior distributions and the conditional posterior distribution of μ are given by

$$\boldsymbol{\Omega_b}|\bar{y}, ss_b, ss_e \sim IG\left(\frac{a-1}{2}, \frac{ss_b}{2}\right) \tag{7}$$

$$\boldsymbol{\Omega_e}|\bar{y}, ss_b, ss_e \sim IG\left(\frac{a(n-1)}{2}, \frac{ss_e}{2}\right) \tag{8}$$

and

$$\boldsymbol{\mu}|\bar{y}, ss_b, ss_e, \Omega_b, \Omega_e \sim N\left(\bar{y}, \frac{\Omega_b}{an}\right) \tag{9}$$

where IG(d,s) is the inverted gamma density with parameters d and s.

Furthermore, if the restrictions did not apply, the joint posterior distribution of $(\mu, \Omega_b, \Omega_e)$ would be the product of these three distributions. But the restrictions $\Omega_b \geq \Omega_e$ do apply. The procedure consists of generating $(\boldsymbol{\Omega_b}, \boldsymbol{\Omega_e})$ from the independent inverted gamma distributions in (6), and (7), and retaining only those pairs $(\boldsymbol{\Omega_b}, \boldsymbol{\Omega_e})$ that satisfy $\Omega_b \geq \Omega_e$ the required Bayesian β-expectation tolerance intervals for $N(\mu, \sigma_b^2 + \sigma_e^2)$ may be estimated with the following algorithm:

Algorithm 1

(1) Choose $T0$, the number of Bayesian simulation, say 10 000;
(2) (a) Generate $T0$ values from the distribution of $(\boldsymbol{\Omega_b}, \boldsymbol{\Omega_e})$ specified in (7) and (8); (b) Retain those sets of values that obey the restricted parameter space $\Omega_b \geq \Omega_e$; Suppose there are T such pairs
(3) Generate T values from the normal distribution of μ given in (9)
(4) For each of the T values of $(\mu, \boldsymbol{\Omega_b}, \boldsymbol{\Omega_e})$, generate a pseudo-random normal observation with mean μ and variance $\sigma_b^2 + \sigma_e^2$
(5) The β_1th and the β_2th percentile of the T values of this pseudo-random normal distribution gives Bayesian two-sided β-expectation tolerance intervals.

Note that:

$$\sigma_b^2 = \frac{\Omega_b - \Omega_e}{n} \quad \text{and} \quad \Omega_e = \sigma_e^2 \tag{10}$$

and

$$\beta = \beta_2 - \beta_1 \tag{11}$$

2.2. Bayesian uncertainty

Since 2000, ISO 17025 requires different sectors such as regulatory bodies, the official quality laboratories the corporation's contractors of services and the industries of various sectors, namely: chemistry, pharmacy, biopharmacy, food processing, environment, cosmetology, etc. to apply procedures for estimating measurement uncertainty [39].

The correct interpretation of a measurement result requires knowledge about its uncertainty. In this context, several approaches for estimating uncertainty in analytical measurements are proposed, the most important ones are:

- The International Organisation for Standardisation (ISO) approach (commonly known as "bottom-up" approach) published in the Guide of Uncertainty of measurement (GUM)

Table 1
Analysis of variance table for balanced one-way random effects model.

Source	Sum of squares	df	Mean square	Expected mean square
Factor	$SS_b = n\sum_{i=1}^{a}(\bar{Y}_i - \bar{Y})^2$	$a-1$	$MS_b = \frac{SS_b}{a-1}$	$n\sigma_b^2 + \sigma_e^2$
Error	$SS_e = \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y}_i)^2$	$a(n-1)$	$MS_e = \frac{SS_e}{a(n-1)}$	σ_e^2
Total	$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{n}(Y_{ij} - \bar{Y})^2$	$an-1$		

[41,42]; The ISO or GUM approach was originally proposed for quantifying uncertainty in physical measurements. It is based on identifying, quantifying and combining all sources of uncertainty on the measurement. This guideline proposes an error-propagation or error-budget approach to estimate the uncertainty related to a measurement result. Nevertheless, direct application of the GUM in analytical, bioanalytical laboratories is found tedious and laborious. Horwitz and Albert [48] states that the error-budget is not generally accepted as the most suitable and practical way to evaluate the uncertainty related to a measurement result in analytical chemistry. For this reason, the EURACHEM guideline was published in order to make a compromise between the requirements of the GUM and the needs of analytical chemists. Indeed, Analytical chemists are used to determining error by what they call 'method validation', and would like to use this for determining uncertainty. When, Metrologists have a different approach, which they apply to physical methods and would like to see applied also in analytical chemistry.

- The Analytical Methods Committee (commonly known as "top–down" approach) [47]. This document has been proposed as an alternative method to evaluate the uncertainty related to each component of an analytical measurement result. The top–down approach is based on precision data assessed in an inter-laboratory study.

Depending on the conditions under which the analyst is operating, different operational definitions of uncertainty have been proposed. They comprise: within-laboratory uncertainty, reproducibility uncertainty, bias-included uncertainty and absolute uncertainty. At this point, we consider the evaluation of the measurement uncertainty derived from the results obtained in a validation experiment.

Although few approaches have been described for the estimation of uncertainty from validation process [27–35], there is still a need to clarify the relationship between validation and uncertainty for many analysts and particularly to show how the validation data can be practically used to estimate the uncertainty measurement. A recent draft of guide ISO/DTS [36] suggests that experimental data obtained from repeatability, reproducibility and trueness studies could be used to determine uncertainty measurement. Furthermore, by considering the validation strategy adopted by SFSTP2003, Feinberg et al. [34] put into practice the possibility to estimate the uncertainty using the validation data and accuracy profiles.

This section will be devoted to expose a new strategy for estimating measurement uncertainty using the Bayesian strategy and validation data.

According to the LGC/VAM protocol [27] and the recommendations of the ISO/DTS 21748 guide [36], a basic model for the uncertainty of the measure and Z, can be expressed by Eq. (12):

$$u^2(Z) = S_R^2 + u^2(\hat{\delta}) + \sum c_i^2 u^2(x_i) \quad (12)$$

where S_R is the reproducibility standard deviation, $u(\hat{\delta})$ is the uncertainty associated with the bias of the method, and $\sum c_i^2 u^2(x_i)$ is the sum of all of the effects due to other deviations.

If the validation data via the accuracy profile are used to estimate measurement uncertainty, the third term of Eq. (12) can be eliminated and uncertainty may be estimated with the following equation:

$$u^2(Z) = S_R^2 + u^2(\hat{\delta}) \quad (13)$$

According to Mee procedure, the β-expectation tolerance interval is equal to:

$$\hat{\mu}_M \pm t(\nu) k \hat{\sigma}_M$$

It can be easily verified that

$$u^2(Z) = k^2 \hat{\sigma}_M^2$$

and a mathematical model to describe the β-expectation tolerance interval is given now by

$$\hat{\mu}_M \pm t(\nu) u(Z) \quad (14)$$

If we assume that Bayesian β-expectation tolerance interval is equal to Mee's formula, the following expression can be used:

$$U_B = \hat{\mu}_M + t(\nu) u(Z) \quad (15)$$

or

$$L_B = \hat{\mu}_M - t(\nu) u(Z) \quad (16)$$

where U_B is upper Bayesian tolerance interval. L_B is lower Bayesian tolerance interval. Finally, the Bayesian Uncertainty can be expressed as:

$$u(Z) = \frac{U_B - L_B}{2t(\nu)} \quad (17)$$

The Bayesian uncertainty calculated using Eq. (17) must be multiplied by an appropriate coverage factor, k, to give the expanded uncertainty $U(Z)$. For normal distribution a coverage factor of 2 gives an interval containing approximately 95% of the distribution of values [37,40].

3. Illustrative examples

In order to demonstrate the applicability of the Bayesian approach, to evaluate the performance of quantitative analytical procedures on the one hand and the assessment of measurement uncertainty on the other hand, we have selected from the bibliography of methods, which use different instrumental techniques such as spectrofluorimetry, liquid chromatography (LC–UV, LC–MS), Capillary electrophoresis and enzyme-linked immunosorbent assay (ELISA).

Table 2
Calculated concentrations of quinine, expressed as mg L^{-1}.

Replicate	Theoretical concentration	Day				
		1	2	3	4	5
1	66	65.33	66.81	67.44	65.72	66.61
2		65.38	66.79	67.48	65.70	66.36
3		65.22	66.72	67.48	65.88	66.70
1	83	84.49	82.83	82.65	82.30	83.74
2		84.53	82.77	82.70	82.51	83.82
3		84.60	82.92	82.56	82.48	83.65
1	100	100.25	101.36	99.98	98.84	99.60
2		100.20	101.44	100.02	98.93	99.77
3		100.32	101.50	99.87	98.75	99.82

Table 3
The observed values of $\bar{Y}$, SS_b and SS_e for the quinine data.

Level	Theoretical concentration	$\bar{Y}$	SS_b	SS_e
1	66	66.3747	8.6631	0.1005
2	83	83.2367	9.3502	0.0679
3	100	100.0433	10.5939	0.072

Table 4
Calculated two-sided β-expectation tolerance intervals of the fluorimetric determination of quinine, using Bayesian approach and Mee's method.

Theoretical concentration (mg L^{-1})	Bayesian β-expectation tolerance limit in mg L^{-1}	Bayesian β-expectation tolerance limit in %
66	[63.97, 68.94]	[−3.07, 4.46]
83	[80.71, 85.86]	[−2.76, 3.45]
100	[97.34, 102.78]	[−2.66, 2.78]
	Mee β-expectation tolerance limit in mg L^{-1}	Mee β-expectation tolerance limit in %
66	[63.80, 68.95]	[−3.33, 4.47]
83	[80.56, 85.92]	[−2.94, 3.51]
100	[97.19, 102.90]	[−2.81, 2.90]

3.1. Fluorescence method for the determination of quinine in tonic water

Gonzalez and Herrador [25] describe and validate a fluorescence spectrometry method for the determination of quinine in tonic water.

According to the Food and Drug Administration (FDA) guidelines, the validation standards were prepared covering approximately the 80%, 100% and 120% of the target amount, 83 mg L^{-1} quinine. Each concentration level was analyzed 3 times ($n = 3$) for 5 days ($a = 5$). The data is given in Table 2.

The fluorescence method will be judged valid if the entire two-sided 95%-expectation tolerance is within the acceptability limits (±5%), an application range of [66, 100] mg L^{-1}.

The Bayesian tolerance interval can be easily estimated by Markov chain Monte Carlo (MCMC) method, and the Algorithm 1 can be easily specified for doing this computation.

We shall compute Bayesian β-expectation tolerance intervals for the distributions $N(\mu, \sigma_b^2 + \sigma_e^2)$ in the context of the fluorescence spectrometry method for the determination of quinine in tonic water, reported in Table 2, using the non-informative prior distributions. In this example, we have the one-way random model and balanced data, with $a = 5$ and $n = 3$. The observed values of $\bar{Y}$, SS_b and SS_e are reported in Table 3:

Using Algorithm 1, we computes a Bayesian two sided 95%-expectation tolerance limit for $N(\mu, \sigma_b^2 + \sigma_e^2)$ using 10 000 simulated samples from the posterior distribution of $(\mu, \Omega_b, \Omega_e)$. Rejection sampling is employed to generate the samples, as pointed out earlier. All results are reported in Table 4

With the aim to calculate the Bayesian measurement uncertainty, we first estimate $t(\nu)$ the β quantile of the student t distribution with ν degrees of freedom using the Satterthwaite approximation [38]. As can be seen from Eq. (17), the Bayesian Uncertainty of the fluorimetric method, is assessed and summarized in Table 5. Note that the expanded uncertainty $U(Z)$ is computed using a coverage factor of $k = 2$.

Table 6
Validation results of the UHPLC method for the determination of folic acid in pharmaceutical preparation using Bayesian approach (acceptance limits $\lambda = \pm 10\%$).

Level	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
1	−0.87	0.64	1.62
2	0.36	0.84	2.06
3	−0.38	0.45	1.47
4	0.48	0.19	0.62
5	0.2	0.16	1.9
	Accuracy		
	Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)	
1	[−5.46, 3.72]	[−5.71, 3.97]	
2	[−5.21, 5.97]	[−5.40, 6.13]	
3	[−4.79, 3.84]	[−4.81, 4.04]	
4	[−1.24, 2.29]	[−1.41, 2.37]	
5	[−4.95, 5.88]	[−5.58, 5.98]	
	Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)	
1	1.78	3.56	
2	2.18	4.36	
3	1.63	3.26	
4	0.66	1.33	
5	1.96	3.92	

Table 5
Bayesian uncertainty of the fluorimetric determination of quinine.

Theoretical concentration (mg L^{-1})	L_B	U_B	$t(\nu)$	$u(Z)$	$U(Z)$ (mg L^{-1})	U (%)
66	63.97	68.94	2.7566	0.901	1.802	2.73
83	80.71	85.86	2.7639	0.932	1.864	2.25
100	97.34	102.78	2.7647	0.984	1.968	1.97

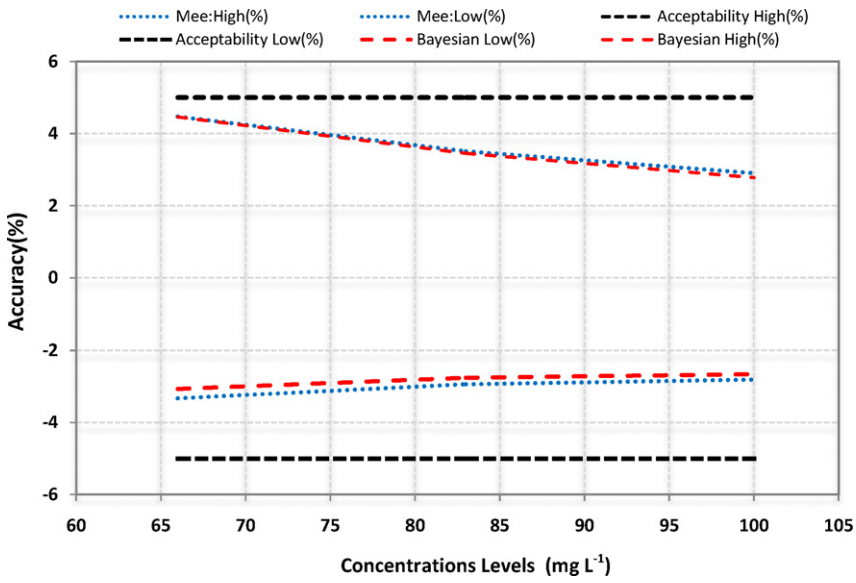

Fig. 1. Two-sided β-expectation tolerance intervals of the fluorimetric determination of quinine, using Bayesian approach and Mee's method. Acceptance limits are set at ±5%.

All results achieved are given in Table 5. As shown in Fig. 1. The Bayesian accuracy profiles are included inside the acceptability limits (5%), at all concentration levels. Accordingly, the method is considered as accurate.

3.2. UHPLC method for the determination of folic acid in pharmaceuticals preparations

Deconinck et al. [43] have described and validated a fully ultra high pressure liquid chromatographic method for qualification and quantification of folic acid in pharmaceutical preparations. After optimization and robustness steps, the authors have been validated their method according to the new SFSTP strategy based on the accuracy profile and the β-expectation tolerance interval. The validation standards at five concentration levels were used. Every sample was prepared in triple and analyzed for five consecutive days. Knowing the sample theoretical concentrations, trueness, repeatability and intermediate precision, the Bayesian tolerance interval has been computed for each concentration level (Table 6). The Bayesian accuracy profile is presented in Fig. 2. As can be seen from the results, the method is valid, since the different tolerance limits did not exceed the acceptance limits (±10%) for all the concentration levels tested.

3.3. SPE-LC–MS method for the determination of cyproterone acetate in human plasma

Christiaens et al. [44] have developed a SPE-LC–MS method for the determination of cyproterone acetate in human plasma. To demonstrate the performance of their method, the authors have validated this procedure according to a new approach using accuracy profiles as a decision tool. In fact, they have adopted the following experimental design $6 \times 3 \times 3 = 54$ that to either the

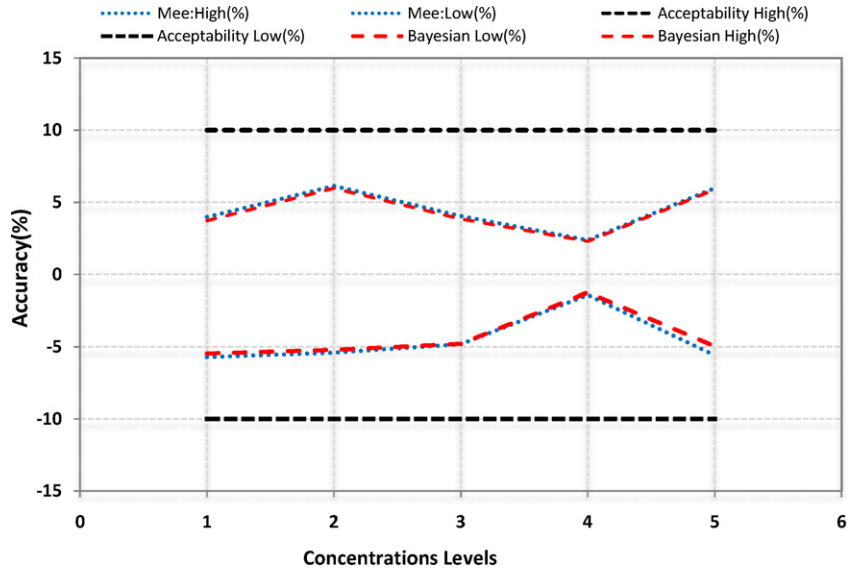

Fig. 2. Accuracy profiles of the UHPLC method for the determination of folic acid in pharmaceutical preparation using Bayesian approach and Mee's method. Acceptance limits are set at ±10%.

Table 7
Validation results of the SPE-LC–MS method for the determination of cyproterone acetate in human plasma using Bayesian approach (acceptance limits λ = ±15%).

Concentration ($ng\,mL^{-1}$)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
0.3	1.2	1.5	1.5
0.5	−0.1	0.9	1.2
1	1.6	1.2	1.6
10	−6.0	0.5	0.5
25	2.5	0.4	0.4
50	4.1	0.4	0.4
	Accuracy		
	Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)	
0.3	[−4.35, 6.99]	[−2.4, 4.7]	
0.5	[−4.49, 4.6]	[−3.4, 3.1]	
1	[−4.57, 7.99]	[−3.2, 6.5]	
10	[−7.94, −4.16]	[−7.2, −4.7]	
25	[0.99,4.02]	[1.6, 3.3]	
50	[2.54, 5.59]	[3.1, 5.1]	
	Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)	
0.3	2.44	4.89	
0.5	1.72	3.44	
1	2.37	4.75	
10	0.82	1.65	
25	0.65	1.31	
50	0.66	1.32	

Table 8
Validation results of the ELISA method for the determination of a neurological disease biomarker protein using Bayesian approach (acceptance limits λ = ±30%).

Concentration ($ng\,mL^{-1}$)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
3.5	14.82	38.41	38.41
7	16.91	17.04	22.69
14.1	6.225	13.47	14.21
28	4.647	4.238	8.229
56	3.246	3.168	6.349
113	3.192	3.191	6.307
225	6.294	4.047	5.696
450	−0.9705	5.185	9.01
	Accuracy		
	Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)	
3.5	[−96.08, 124.48]	[−66.33, 95.97]	
7	[−51.37, 85.48]	[−37.22, 71.05]	
14.1	[−35.22, 47.75]	[−24.3, 36.75]	
28	[−20.71, 29.89]	[−18.64, 27.93]	
56	[−16.97, 23.14]	[−14.93, 21.42]	
113	[−16.62, 22.99]	[−14.78, 21.16]	
225	[−11.03, 23.41]	[−7.678, 20.27]	
450	[−26.66, 29.26]	[−25.35, 23.41]	
	Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)	
3.5	49.99	99.99	
7	28.75	57.50	
14.1	18.66	37.32	
28	9.49	18.97	
56	7.39	14.78	
113	7.33	14.65	
225	7.09	14.19	
450	10.74	21.48	

validation standards or the calibration standards. The tolerance probability β was set at 95% and the acceptance limit at ±15%.

By application of Bayesian approach, the validation results of the response function are presented in Table 7. A weighted linear regression ($1/\chi^2$) with six concentration levels was used and its goodness of fit is illustrated in Fig. 3. As can be seen from the results in Table 7, the different limits of Bayesian tolerance (the upper and lower β-expectation tolerance limits) do not exceed the acceptance limits settled at ±15% for each concentration level.

3.4. ELISA method for the determination of a neurological disease biomarker protein

In this example we consider the enzyme-linked immunosorbent assay (ELISA) presented in the new guide SFSTP2003 [45]. ELISA test has been developed in order to exactly quantify a protein likely to be a biomarker and used for a neurological disease-related therapeutic project. To validate this technique, the calibration standards and the validation standards were prepared in appropriate matrices, from protein stock solutions by serial dilution in four independent series, with two plates by trial, over 4 days. All the trials – either for calibration or validation standards – have been conducted in triplicates. As response function, the Commission had selected the logistic model with four parameters.

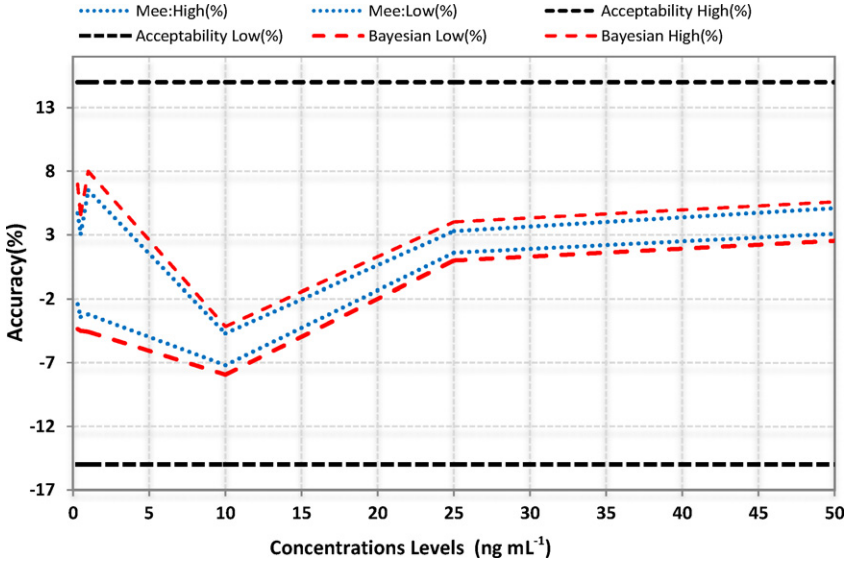

Fig. 3. Accuracy profiles of the SPE-LC–MS method for the determination of cyproterone acetate in human plasma using Bayesian approach and Mee's method. Acceptance limits are set at ±15%.

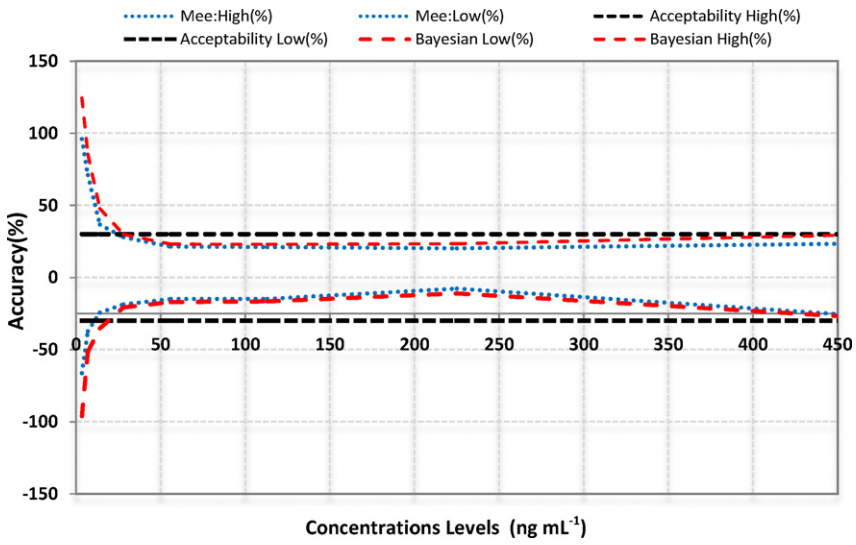

Fig. 4. Accuracy profiles of the ELISA method for the determination of a neurological disease biomarker protein using Bayesian approach and Mee's method. Acceptance limits are set at ±30%.

Since we have sufficient statistics, we performed the validation of ELISA test and the estimation of measurement uncertainty.

All results achieved are given in Table 8. As shown in Fig. 4. The Bayesian accuracy profiles are included inside the acceptability limits (30%), at all concentration levels, except at levels lower than 28 ng mL^{-1}. Consequently, the method can be considered as giving accurate results between 24 and 450 ng mL^{-1}.

3.5. *Capillary electrophoresis method for the determination of quinine, furosemide and the combination trimethoprime/sulfamethoxazole*

Reliable low-cost capillary electrophoresis device for drug quality control and counterfeit medicines has been proposed by Marini et al. [46]. Indeed, the electrophoretic technique has been applied for the determination of quinine, furosemide and the combination trimethoprime/sulfamethoxazol in the medicament forms.

The method validation has been performed according to the recommendations of the SFSTP2003 regarding total error concept and the last FDA proposal. The selectivity, trueness, repeatability and intermediate precision have been evaluated on four different validation series. Accuracy profile based on tolerance intervals was used to select the best calibration function and to determine the validated concentration ranges. The tolerance probability β has been set at 95% and the acceptance limit at ±10%.

The upper and lower Bayesian tolerance limits expressed in relative value (%) are presented in Figs. 5–8 as a function of the introduced concentrations. As can be seen from the results (Table 9), the method is considered as accurate, since the tolerance intervals are included in the ±10% acceptance limits for all the concentration levels tested except the lowest one (80% for sulfamethoxazole).

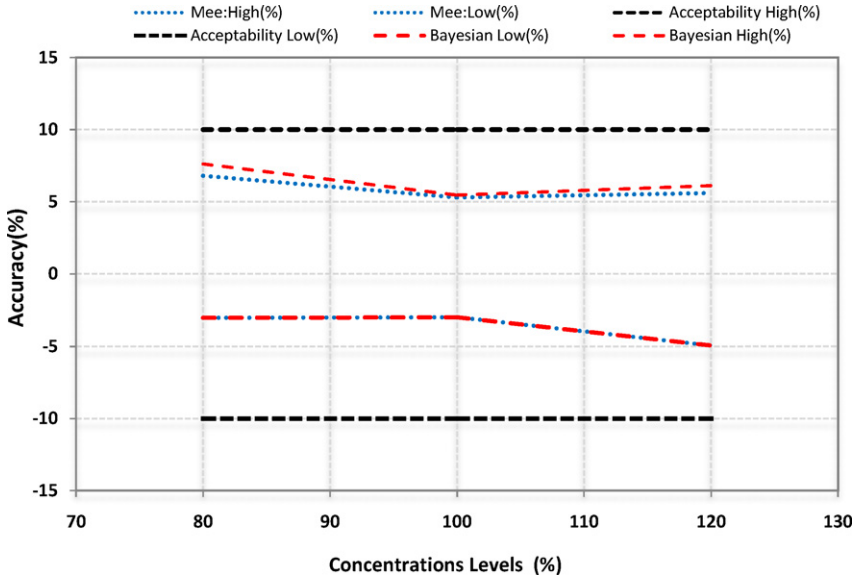

Fig. 5. Accuracy profiles of the EC method for the determination of quinine in pharmaceutical preparation using Bayesian approach and Mee's method. Acceptance limits are set at ±10%.

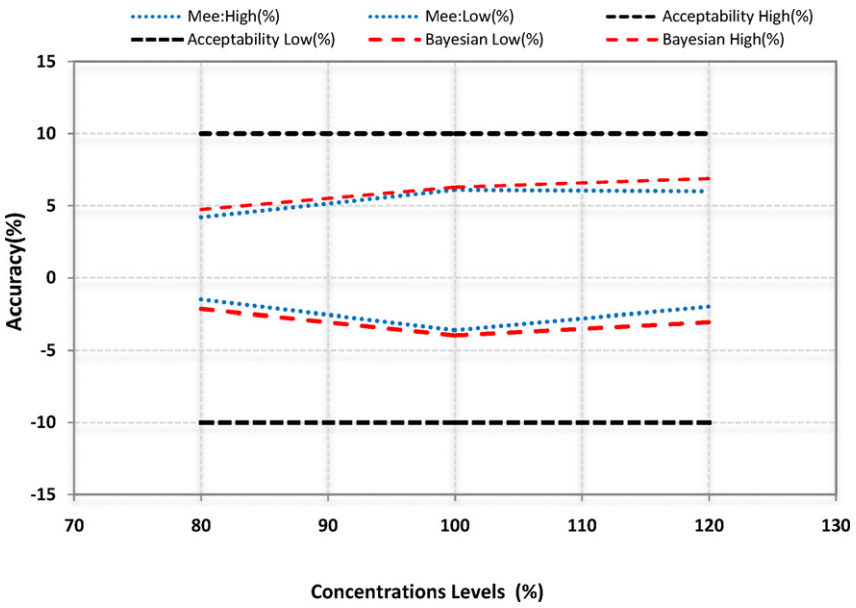

Fig. 6. Accuracy profiles of the EC method for the determination of furosemide in pharmaceutical preparation using Bayesian approach and Mee's method. Acceptance limits are set at ±10%.

4. Discussion

According to the intervals assembled in Tables 4–9, we can state that the quantitative analytical and bioanalytical methods that have been the subject of this study as valid, since the two sided β-expectation tolerance limit estimated, by either the Bayesian approach or Mee's method fall inside the acceptance limits. Moreover, we have found that intervals calculated by the conventional method adopted by SFSTP2003 [16] and the Bayesian strategy are almost the same. Through the selected examples, we have demonstrated clearly that the proposed approach is applicable to assess the performance of analytical procedures which can use different instrumental techniques such as spectrofluorimetry, liquid chromatography (LC–V, LC–S), Capillary electrophoresis and enzyme-linked immunosorbent assay (ELISA). Also, it is noted that we have the opportunity to use various calibration models even non linear, like four-parameter logistic model frequently applied to ELISA techniques. Indeed, the objective of an analytical procedure is to give accurate measurements. Accordingly a calibration curve must be evaluated on its ability to provide accurate measurements. A significant source of bias and imprecision in analytical measurements can be caused by the inadequate choice of the statistical model for the calibration curve. For this reason, we have introduced the use of the accuracy profile based on the Bayesian tolerance intervals to decide if a calibration model will give results of sufficient quality. The models should be retained or rejected based on the accuracy of the back-calculated results regardless of the statistical properties. Throughout the chosen examples we can state that, the proposed strategy can support all possible response functions. Only requires that the selected model is able to provide accurate results. This can be translated by the Bayesian accuracy limits which fall totally within the acceptance limits.

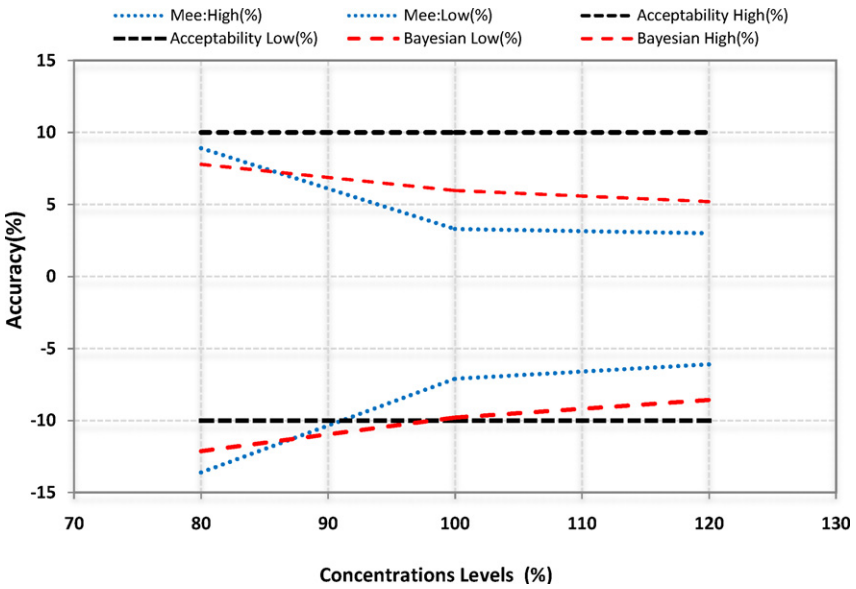

Fig. 7. Accuracy profiles of the EC method for the determination of sulfamethoxazole in pharmaceutical preparation using Bayesian approach and Mee's method. Acceptance limits are set at ±10%.

Table 9
Validation results of the EC method for the determination of quinine, furosemide, sulfamethoxazole and trimethoprime in pharmaceutical preparation using Bayesian approach (acceptance limits $\lambda = \pm 10\%$).

Quinine				Sulfamethoxazole			
Concentration (%)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)	Concentration (%)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
80	2.3	2	2	80	−2.3	1.7	2.5
100	1.2	1.2	1.5	100	−1.9	2.1	2.1
120	0.6	1.4	1.9	120	−1.6	1.8	1.6
	Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)			Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)	
80	[−3.02, 7.62]	[−2.3, 6.8]		80	[−12.12, 7.79]	[−13.6, 8.9]	
100	[−2.98, 5.46]	[−2.9, 5.3]		100	[−9.79, 5.97]	[−7.1, 3.3]	
120	[−4.93, 6.11]	[−4.4, 5.6]		120	[−8.57, 5.20]	[−6.1, 3.0]	
	Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)			Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)	
80	2.49	4.99		80	3.56	7.12	
100	1.87	3.74		100	3.39	6.79	
120	2.38	4.75		120	2.85	5.70	
Furosemide				Trimethoprime			
Concentration (%)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)	Concentration (%)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
80	1.3	1.2	1.2	80	1.9	1.8	1.8
100	1.2	1.2	1.7	100	1.6	1.2	1.2
120	1.9	1.6	1.7	120	0.8	0.8	0.8
	Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)			Bayesian expectation tolerance interval (%)	Mee expectation tolerance interval (%)	
80	[−2.15, 4.74]	[−1.5, 4.2]		80	[−4.77, 8.66]	[−2.6, 6.3]	
100	[−3.96, 6.28]	[−3.6, 6.1]		100	[−2.96, 6.14]	[−1.5, 4.6]	
120	[−3.05, 6.88]	[−2.0, 6.0]		120	[−2.27, 3.79]	[−1.1, 2.8]	
	Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)			Bayesian uncertainty (%)	Bayesian expanded uncertainty (%)	
80	1.56	3.12		80	2.89	5.79	
100	2.11	4.21		100	1.96	3.92	
120	2.23	4.46		120	1.31	2.61	

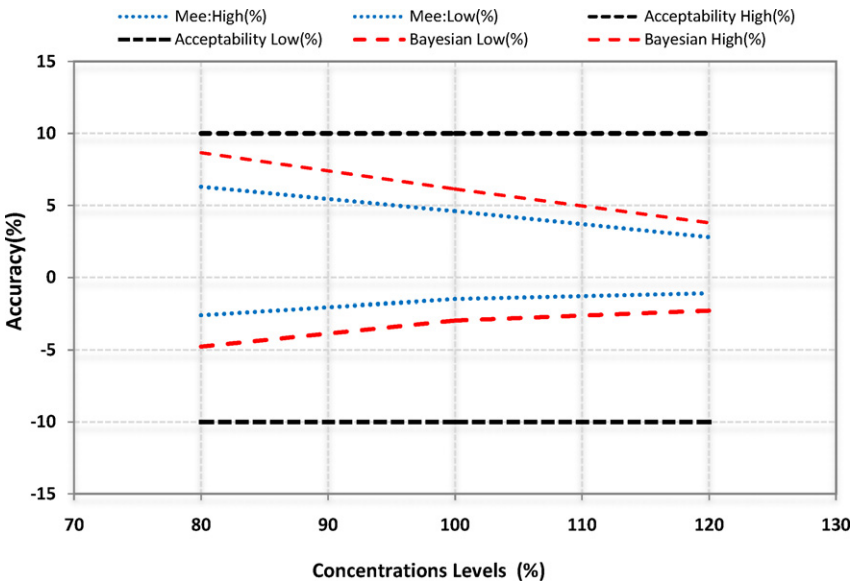

Fig. 8. Accuracy profiles of the EC method for the determination of trimethoprime in pharmaceutical preparation using Bayesian approach and Mee's method. Acceptance limits are set at ±10%.

Most importantly, the measurement uncertainty is as an essential subject as analytical validation or even more. In fact, the estimation of measurement uncertainty is considered the major problem for laboratories because it requires a higher degree of technicality and mastery of statistical tools. In order to assist analysts in this direction, we have developed an original formula based on the Bayesian β-expectation tolerance interval for assessing uncertainty. One major advantage of the proposed methodology is that it can, without any additional experiments, give an estimation of uncertainty of measurements based on the information from validation stage. In this way, the data used to carry out the validation using the accuracy profiles have also been used for the estimation of measurements uncertainty. Indeed, the uncertainty is derived from the Algorithm 1 used to construct the Bayesian β-expectation tolerance limits. On this basis, several uncertainty results were generated and are presented in Tables 4–9. It must be reminded that the expanded uncertainty has been computed using a coverage factor of $k=2$ representing an interval around the results where the unknown true value can be observed with a confidence level of 95%.

In light of the obtained results, we can conclude that the Bayesian approach can be applied to estimate the tolerance intervals, and, consequently validate the quantitative analytical and bioanalytical procedures. We believe that the proposed approach is an effective tool which can be used as a fitness of purpose criterion of measurement methods as well as to give a good estimate of the measurement uncertainty.

The Bayesian accuracy profile procedure allows us without difficulty to evaluate the capability of an analytical method to quantify samples with a known accuracy and a fixed risk according to that method's objective.

Compared to the classical strategy, the new proposal has a more holistic character. It means that it is no longer necessary to know the various individual steps into which the analytical method can be broken down since the analytical procedure is taken as a whole. It is not necessary to know the bias and reproducibility of the method for assessing uncertainty. Not only does this approach simplify the validation process of an analytical method, but also allows the monitoring of risk associated to its employment.

5. Conclusion

In this paper, we have managed to implement a new technique to validate the quantitative analytical and bioanalytical procedures based on the Bayesian simulation and accuracy profiles. As well, an original method for estimating measurement uncertainty by the same approach is proposed. By means the selected examples, we have indeed demonstrated the possibility to apply our concept to diverse activity sectors such as pharmacy, biopharmacy or food processing.

In conclusion, this strategy offers the opportunity to validate the analytical procedure as well as to assess its uncertainty in the same way in which it will be used in the routine. It is therefore possible with the Bayesian tolerance interval to minimize considerably the risk to accept a procedure that would not be sufficiently accurate or, on the contrary, to reject a procedure that would be appropriate.

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