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Assessing the Validity of an LC-MS/MS Method Using Uncertainty Profile as Decision Criterion to the Determination of Nevirapine in Human Plasma

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ABSTRACT

The full validity of an analytical method requires a good awareness of the tools available, and is therefore a major asset in guiding laboratories towards the adoption of appropriate approaches, not only to enable both analytical validation and estimation of measurement uncertainty, but also to make less effort and experience. Indeed, a graphical approach namely uncertainty profile intended to assess the validity and estimate the measurement uncertainty of analytical procedure has been applied, its success reside to the construction of the β-content, γ-confidence tolerance interval.

The purpose of this paper is to describe and test a full validation of an LC-MS method for the determination of nevirapine in human plasma using the uncertainty profile as a new holistic validation approach. By exploiting data collected under intermediate precision conditions, we have come up with a good assessment of the uncertainty of chemical measurements through an innovative formula, based on the β-content tolerance interval without referring to any extra effort and additional experiments. Three statistical procedures, namely: Satterthwaite approximation, the generalized pivotal confidence method and the modified large simple procedure have been used to build this tolerance interval.

Regardless of the statistical model tested, the proposed analytical procedures were validated over the selected validation domains since 66.7% of the future results falls inside the acceptance limits of ± 15%.

Keywords: Method validation; Uncertainty profile; Nevirapine; Tolerance interval; LC-MS

INTRODUCTION

The ultimate objective of good laboratory practice is to ensure the reliability of the analytical results. This concept defined as "Good Laboratory Practice (GLP) refers to the organizational process and the conditions under which the analysis and tests are planned, executed, monitored, recorded and reported [1]. Actually, the analytical validation and estimation of measurement uncertainty are commonly used in chemical analysis [2-5]. They are mandatory steps in the life cycle process of an analytical method, and are used to improve analytical and regulatory requirement for routine analysis [6].

In fact, various guidelines of analytical validation and uncertainty measurement have been proposed by many regulatory bodies in order to help analysts to take a good decision about their analysis report [7-12]. However, these normative documents often lead to different explanations and obviously to contradictory conclusions. In addition, they do not propose an experimental protocol to conduct validation in practice; they are often limited to general concepts and to the definition of performance criteria for analytical methods. Therefore, sometimes these guidelines very often create confusion in the denomination of certain performance criteria.

Looking at the current situation with regards to the different validation approaches and their statistical bases, we can classify them into two groups:

(1) The conventional strategies based on the statistics of the null hypothesis and the separate evaluation of fidelity and trueness [13-15].
(2) The newer strategies based on tolerance interval statistics and accuracy [16,17].
Although the classical approaches are habitually used to evaluate the validation of analytical methods particularly in pharmaceutical and biopharmaceutical industry, they have proved to be flawed as practice has pertinently revealed. These short comings reside in the separate evaluation of the systematic error (bias) and random error (variance) [18]. Indeed, this way of considering the performance of analytical methods generates two major problems:
(1) The impossibility of adjustment of a bigger variance to a smaller bias (and reciprocally).
(2) The total or partial unbalancing between client risk and laboratory risk. i.e., The risk to declare that an analytical validation is not acceptable when in reality it is. Or else, the risk to declare that an analytical validation is acceptable when in reality it is not.
On the other hand, in order to conclude once and for all on the reliability of the analytical results, alongside the validation it is of paramount importance to evaluate the uncertainty of measurement. Because, this parameter is indispensable in the interpretation of the chemical measures and, especially, in the comparison with regulatory limits of conformity [19, 20].
In parallel, most bioanalytical method need a special intention when a given study is conducted at more than one site, it is necessary to validate the bioanalytical method(s) at each site and provide appropriate validation information for different sites to establish interlaboratory reliability [21].
Hence, It is essential to employ well-characterized and fully validated bioanalytical methods under certification and accreditation standards such as ISO 17025 and 15189, it is also recommended for testing laboratories to apply procedures for estimating uncertainty and to be able, if necessary, to be associated with the results returned [8].
Despite the fact that several standards and guidelines of assessment of uncertainty have emerged since then, the implementation of this concept in the quality assurance system remains the major problem for the laboratories. This problem stems from the incapability of analytical chemists to apply current metrological standards. Indeed, the construction of the uncertainty-budget model as recommended by ISO-GUM and EURACHEM is extremely challenging in the case of measurements with analytical and bioanalytical methods.
In response to this situation, researchers have recently proposed innovative formulas for the estimation of measurement uncertainty from data collected under intermediate precision conditions [22]. This way of doing things, is a more comprehensible approach than the uncertainty-budget concept and thus will be easily integrated.
Nowadays, newfangled validation strategies based on the tolerance interval and accuracy are an interesting alternative for laboratories to implement measurement uncertainty in their quality assurance system. Through these holistic strategies, it is possible to estimate the measurement uncertainty from the validation data without resorting to additional experiments [22-27].
In the framework of the fit for purpose of analytical method, the purpose of this paper is to describe and explain a full validation of an LC-MS method for the determination of nevirapine (NVP) in human plasma by using uncertainty profile as a newfangled holistic approach, exploiting three different statistical methodologies to build the tolerance interval.
Furthermore, different gamma and beta values have been tested for the estimation of measurement uncertainty in order to better interpret the reliability of the analytical results generated by this LC-MS method.
Ultimately, head-to-head comparison of measurement uncertainties provided by different total error approaches "the β-content, γ-confidence tolerance interval and β-expectation tolerance interval" of this method has been investigated.

METHODS

Analytical Procedure

We aimed to assess the implementation of our new holistic approach on a bioanalytical method, through the determination of NVP in human plasma by LC/MS [28].
Protein precipitation technique was used for the determination of NVP in human plasma samples. Optimal extraction was performed using NVP-d4 as an internal standard. Applying the Atmospheric pressure ionization API2000, the samples were ionized by positive ions ESI+ in an electrospray mode.

Calibration Standards Solutions

Calibration standard solutions were prepared by spiking human plasma with four concentration levels of (k=4) 30, 90, 1000 and 2500 ng.mL-1. They were replicated six times (p=6) for five daily independent preparation (n=5).

Hence, the total number of calibration standard measures is 120 which are sufficient to establish the response functions.

Validation Standards Solutions

As regards the validation standard solutions, they were obtained from stock solutions prepared independently from a batch of homogenized human plasma and spiked with NVP-d4 at the same concentration levels of calibration standards (k=4). The analysis of solutions has been replicated six times (p=6) for five days (n=5). Thus, the total number of validation standard measures is 120 that is sufficient to construct the uncertainty profile.

Uncertainty Profile Theory

The uncertainty profile is a decision tool for analytical methods, it not only allows to validate a method, but it also opts for the estimation of its uncertainty. Indeed, it constitutes a real graphic tool, intended to measure the reliability of the future results, it is based on the integration of uncertainty limits within the acceptance limits. It is constructed by the tolerance intervals (the prediction interval) β-content.

This work is devoted to the validation of a new LC-MS method, for the determination of NVP, in a human plasma matrix.

A comparative study was carried out in the framework of this work, to verify the flexibility of the profile in the estimation of measurement uncertainty.

β-content Tolerance Interval

The tolerance interval is a prediction interval; it ensures the reliability of future results with a given probability, moreover it is a mathematical tool used in the calculation of the limits of uncertainties. In order to show its usefulness, three Chemometric approximations were applied. Similarly, a comparative study of the different methods will be undertaken, in order to examine their possibilities of providing identical results [29].

Satterthwaite approximation (Mee)

Approximation, including the estimation of variances, is a well-known. Indeed, the Mee approach allows the determination of an effective degree of freedom of a distribution whose probability is formed by several independent normal distributions [30].

The tolerance interval according to the method of Mee can be schematized by the following relation:

$$\bar{Y} \pm k_s \hat{\sigma}_m \qquad (1)$$

Where

$$\hat{\sigma}_m{}^2 = \hat{\sigma}_b{}^2 + \hat{\sigma}_e{}^2 \qquad (2)$$

$\hat{\sigma}_m{}^2, \hat{\sigma}_b{}^2 and\ \hat{\sigma}_e{}^2$ the estimates of the reproducibility variance, the between condition variance and the within condition variance (repeatability).

With

$$k_s \approx \left(\frac{f \chi_{1;\beta}{}^2(h)}{\chi_{f;1-\gamma}{}^2} \right)^{\frac{1}{2}} \qquad (3)$$

And

$$f = \frac{(R+1)^2}{\frac{(R+n^{-1})^2}{(a-1)} + \frac{(1-n^{-1})}{(an)}} \qquad (4)$$

Or

$$R = max\left[0, {}^{(FF_n - 1)}/_{n}\right] and\ R_0 = \frac{R+1}{nR+1} \qquad (5)$$

And

$$h = \frac{1}{anR_0} \quad (6)$$

Where

F is the mean square ratio MSb/MSe and Fh is the 100h percentile of an F distribution with υ_1= a (n-1) and υ_2=a-1. However, based on numerical results, the recommended values of η=0.85, 0.905 and 0.975, corresponding to γ=0.90, 095 and 0.99, respectively.

$\chi_{1;\beta}{}^2(h)$denotes the b quantile of a non-central chi-square distribution with degrees of freedom f and non-centrality parameter h.

a is the number of series.

n is the number of independent replicates per series.

Liao–lin–iyer approach

The calculation of tolerance interval β-content, with the approach of Liao et al, is performed with the Monte-Carlo simulation in the form of an algorithm [31, 32], the steps are:

At the beginning, we calculate the values of these various expressions $\bar{Y}$, SSb et SSe ;

We note M: the number of stimulations, for i=1,2 … M, then we apply the following stages:

We generate independent random variables: $A^2_{b,i} \sim \chi^2_{a-1}$ et $A^2_{e,i} \sim \chi^2_{a(n-1)}$.

Then, we calculate:

$$L_{1,i} = \frac{\left(\frac{1}{n}\right)\left(1+\frac{1}{a}\right)ss_b}{A^2_{b,i}} + \frac{\left(1-\frac{1}{n}\right)ss_e}{A^2_{e,i}} \quad (7)$$

The upper bound of the confidence interval is derived from the quantile γ of L_1, i.

The square root of the upper bound of the confidence interval will provide a statistical error margin D, which will then constitute a basis for the calculation of the tolerance interval.

Once the value of D is obtained, the calculation equation of the tolerance interval is as follows:

$\bar{Y} \pm D$ (8)

Hoffman–Kringle

The method proposed by Hoffman-Kringle applies to the construction of tolerance intervals for random effects models. It is applied when a data set is balanced or not [33].

To illustrate this method in a balanced one-way random model, we define:

$$\sigma_1^2 = \sigma_b^2 + \sigma_e^2 \; and \; \sigma_2^2 = \frac{n\sigma_b^2+\sigma_e^2}{an} \quad (9)$$

Since equation (9), we write:

$$\sigma_1^2 + \sigma_2^2 = \left(1+\frac{1}{a}\right)\frac{n\sigma_b^2+\sigma_e^2}{an} + \left(1-\frac{1}{n}\right)\sigma_e^2 \quad (10)$$

The confidence upper bound of the MLS procedure is given by the following relation:

$$S = \left[\left(1+\frac{1}{a}\right)\frac{n\sigma_b^2+\sigma_e^2}{an} + \left(1-\frac{1}{n}\right)\sigma_e^2\right] + \left[\left(1+\frac{1}{a}\right)^2\frac{\left(n\sigma_b^2+\sigma_e^2\right)^2}{n^2}\left(\frac{a-1}{\chi^2_{a-1;1-\gamma}}-1\right) + \left(1-\frac{1}{n}\right)^2\sigma_e^4\left(\frac{a(n-1)}{\chi^2_{a(a-1);1-\gamma}}-1\right)^2\right]^{0.5} \quad (11)$$

The tolerance interval with γ confidence level is calculated by the following relation:

$$\bar{Y} \pm Z_{(1+\beta)/2}\sqrt{S} \quad (12)$$

Uncertainty Computation

The measurement uncertainty estimation according to ISO / DTS 21748, GUM and Eurachem is given by the following equation [11, 19, 34]:

$$u^2(Z) = S_R^2 + u^2(\hat{\delta}) + \sum c_i^2 u^2(x_i) \qquad (13)$$

Where

S_R : the reproducibility standard deviation ;

$u(\hat{\delta})$: the uncertainty associated with the bias of the method;

$\sum {C_i}^2 u^2(x_i)$: the sum of all of the effects due to other deviations.

According to Feinberg et al. the third term in equation (13) is neglected; its effect is smaller than the main components of uncertainty. Therefore, the equation of uncertainty becomes:

$$u^2(Z) = S_R^2 + S_{\bar{Y}}^2 \ (14)$$

We know that:

$$S_{\bar{Y}} = S_r\sqrt{\frac{1}{IJ\times B^2}} \ and \ B = \sqrt{\frac{Q+1}{J\times Q+1}} \ (15)$$

The term Q is equal to the interlaboratory variance on the variance of repeatability, when we replace the term relative to the bias $S\bar{y}$ by its expression the equation of uncertainty $u^2(Z)$ becomes as follows:

$$u^2(Z) = S_R^2 + \left({S_r}^2 \times \left(\sqrt{\frac{1}{IJ\times B^2}}\right)^2\right) = K^2 \times S_R^2 \qquad (16)$$

It is known that the tolerance interval β-expectation uses the present equation:

$$\bar{Y} \pm t(\vartheta) k \hat{\sigma}_M \qquad (17)$$

We know that σˆ_M is equal to SR, so we can easily show that:

$$u^2(Z) = K^2 \hat{\sigma}_M^2 \ (18)$$

Thus, the mathematical model that brings together the measurement uncertainty and the tolerance interval is expressed by the following relation:

$$\bar{Y} \pm t(\vartheta) u(Z) \qquad (19)$$

If we develop equation (19), the limits of uncertainty will be as follows:

$$U = \bar{Y} + t(\vartheta) u(Z) \qquad (20)$$

And

$$L = \bar{Y} - t(\vartheta) u(Z) \qquad (21)$$

Where

U: the upper tolerance limit;

L: the lower tolerance limit.

Finally, the expression of the measurement uncertainty becomes in the following form:

$$u(Z) = \frac{U-L}{2t(\vartheta)} \quad (22)$$

Where t(ϑ) is the(1+γ)/2 quantile of Student t distribution with υ degrees of freedom.
Otherwise, Saffaj et al, proposed an equation to construct the uncertainty profile, which will be used as decision tools for analytical validation.

$$|\bar{Y} \pm ku(Z)| \leq \lambda \quad (23)$$

Where

k : the coverage factor. The choice of the factor k is based on the level of confidence desired. For an approximate level of confidence of 95%, k is equal to 2.

$\bar{Y}$: the estimate of the mean results;

λ : the acceptance limits.

Also the uncertainty profile can be expressed by the following equation:

$$|Biais \pm ku(Z)| \leq \lambda \quad (24)$$

RESULT

The evaluation of the uncertainty profiles indicates that the simple linear function is the appropriate model for NVP. Indeed, this examination was preceded by the determination of several regression models as well as the calculation of the predicted inverse concentrations. These statistics were used to construct the uncertainty profile. The results are shown in (Table 1).

According to (Table 1), it is noted that 95% of predicted results do go beyond the acceptance limits of ± 15%, likewise for uncertainty limits. However, when the risk percentage was changed to 90% for γ and β, the profile was validated for a risk threshold of ± 15%, but when the acceptance limits are reduced to ± 5%, the substances remains within the standards at risk of 66.7%, as shown in (Figure 1), in fact several slip results become non-compliant.

Making a decision on the validity of the method depends mainly on the risk compromise arranged between customers and suppliers. The uncertainty profile graphic (Figure 1) for the NVP shows uncertainty limits, acceptance limits and the range of concentration**.**

In parallel, three Chemometric methodologies are exposed to build the (β, γ) tolerance interval, namely: the Satterthwaite approximation, the GPQ method (generalized pivotal confidence) and the MLS procedure (modified large simple).

Several values of β and γ were chosen. The results are shown in Table 1.

Subsequently, the uncertainty profile suggests the uncertainty calculation whose results have been reported in Table 2. The coverage factor used for the Calculation of the extended uncertainty is equal to 2, to establish a 95% confidence interval where the measurement results have a probability of occurrence.

The results presented in Table 2 indicate that the method has been successfully validated.

An example for the calculation of the uncertainty limits was performed for the first concentration level of NVP (30 ng / mL) using the three approaches cited above.

In this experiment we have $a = 6$ and $n = 5$, $SS_b = 1.5116$ and $SS_e = 0.7694$.

Table 1: Uncertainty limits of nevirapine using different values of β-content and γ-confidence tolerance intervals

Compound	Concentration levels (ng/ mL)	Uncertainty Limits (%)	β	γ
Nevirapine	30	[-5.04, 5.28]	0.667	0.9
	90	[-4.28, 3.91]		
	1000	[-2.39, 3.47]		
	2500	[-2.80, 3.12]		

	30	[-8.63, 8.87]	0.9	0.9
	90	[-7.14, 6.77]		
	1000	[-4.44, 5.51]		
	2500	[-4.86, 5.18]		
	30	[-10.3, 10.54]	0.95	0.9
	90	[-8.47, 8.10]		
	1000	[-5.39, 6.47]		
	2500	[-5.81, 6.14]		

Table 2: Analytical validation results of nevirapine. (Acceptance limits λ=±15%)

Substance	Concentration levels	Relative Bias (%)	Repeatability (RSD%)	Intermediate precision (RSD%)	Relative uncertainty limits	Relative expanded uncertainty (%)	Decision
Nevirapine	1	0.121	2.923	3.15	[-10.3, 10.54]	10.421	Valid
	2	-0.186	2.903	2.903	[-8.47, 8.10]	8.29	Valid
	3	0.536	2.003	2.003	[-5.39, 6.47]	5.935	Valid
	4	0.161	1.58	1.755	[-5.81, 6.14]	5.981	Valid

Table 3: Measurement uncertainty provided by two methods: (β-content, γ-confidence tolerance interval) (γ=95%, β=95%) and (β-expectation tolerance interval) (β=95%)

Relative expanded uncertainty (%)						
Substance	Concentration levels	β-content, γ-confidence tolerance interval	β	γ	β-expectation tolerance interval	β
Nevirapine	1	10.421	0.95	0.9	6.475	0.95
	2	8.29			5.902	
	3	5.935			4.072	
	4	5.981			3.624	

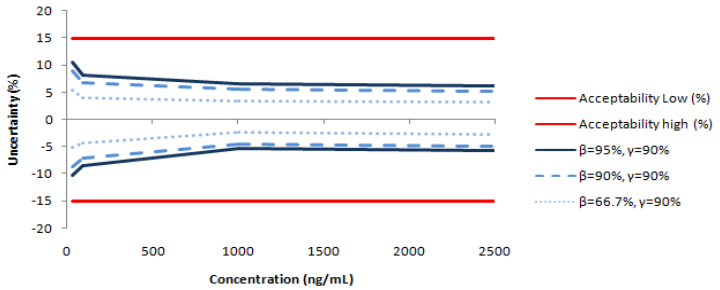

Figure 1: Uncertainty profiles of LC-MC method for the assay of nevirapine in human plasma matrix using Mee method. Acceptance limits are set at λ= ±15%

Mee's approach: method 1

Since $\gamma = 0.9, \eta = 0.905$.The F critical value to compute R_0 is $F_{5,6;1-\eta} = 0.3376$, $R = 0.8033$, $R_0 = 0.3099$. Furthermore, $f = 12.3657$. The appropriate chi-square quintile can be easily obtained from a statistical software package, and are as follows:

$\chi^2_{1;\beta}(h) = \chi^2_{1;0.0667}\left(\frac{1}{9.2}\right) = 1.0417$, and $\chi^2_{f;1-\gamma} = 6.5723$.

Thus, the tolerance factor is equal to:

$$K_s \approx \sqrt{\frac{f\,\chi^2_{1;\beta}(h)}{\chi^2_{f;1-\gamma}}} = \sqrt{\frac{12.36\times 1.0417}{6.5723}} = 1.4$$

And the (0.667, 0.90) tolerance interval is $\left[\bar{Y} \pm k_S \hat{\sigma}_m\right] = \left[28.71, 31.35\right]$. After, we can estimate the measurement uncertainty using eq. (21):

$$u(\mathrm{Y}) = \frac{U-L}{2t(\upsilon)} = \frac{31.35-28.71}{2\times 1.7090} = 0.773\, ng/mL$$

The two sided uncertainty result interval is given by $\left(\bar{Y} \pm 2\times u(\mathrm{Y})\right) = \left(28.49, 31.58\right)$. Equivalently, the tolerance interval can also be expressed by the following relative interval $\left(-5.04\%, 5.28\%\right)$ from the analysed concentration of (30 ng/mL).

Liao-Lin-Iyer approach: method 2

To apply this approach, we estimated a 90% generalized upper confidence limit as1.9245. Monte Carlo simulation consist of 10 000 runs was applied to algorithm presented before to get this limit. Noting that the standard normal quantile $Z_{(1+\beta)/2} = Z_{0.8335} = 0.9681$, we found $D = Z_{0.8335} \times \sqrt{1.8993} = 1.343$.

Thus, the (0.667, 0.90) tolerance interval is given by:

$\left[\bar{Y} \pm D\right] = \left[30.03 \pm 1.343\right] = \left[28.70, 31.37\right]$

A measurement uncertainty can then be calculated using eq (21):

$$u(\mathrm{Y}) = \frac{U-L}{2t(\upsilon)} = \frac{31.37-28.70}{2\times 1.70} = 0.78\, ng/mL$$

The resulting two sided uncertainty interval is given by $\left[\bar{Y} \pm 2\times u(\mathrm{Y})\right] = \left[28.46, 31.60\right]$.Equivalently, the interval is $\left[-5.11\%;\ 5.36\%\right]$ from the analysed concentration of (30 ng/mL).

Hoffman–Kringle approach: method 3

We shall now illustrate the application of the MLS methodology. For $\gamma = 0.9, a = 6$ and $n = 5$ we have $\chi_{a-1;1-\gamma}{}^2 = \chi_{5;0.1}{}^2 = 1.063$ and $\chi_{a(n-1);1-\gamma}{}^2 = \chi_{24;0.1}{}^2 = 16.47$. The above expression for the 90% MLS upper confidence limit simplifies to $S = 1.8417$.

Furthermore, the (0.667, 0.90) tolerance interval is $\left[\bar{Y} \pm Z_{(1+\beta)/2}\sqrt{S}\right] = \left[28.72, 31.35\right]$.

After, we can estimate the measurement uncertainty using eq (21):

$$u(\mathrm{Y}) = \frac{U-L}{2t(\upsilon)} = \frac{31.35-28.72}{2\times 1.70} = 0.768\, ng/mL$$

The resulting two sided uncertainty interval is given by $\left[\bar{Y} \pm 2 \times u(Y)\right] = \left[28.49, 31.56\right]$. Equivalently, the interval is $\left[-5.0\%, 5.25\%\right]$ from the analysed concentration.

DISCUSSION

Previously, analytical validation was an inescapable means of designing and improving new analytical procedures, it was also used to justify the quality of future results. Today, it is an insufficient step to deal with the regulatory requirements: to characterize the performance of the methods, as well as to facilitate the interpretation of the results, therefore, measurement uncertainty must be known.

Although it appears that the use of metrological uncertainty approaches (GUM and EURACHEM approaches) is essential, but sometimes the daily practice of laboratories do not resist the complexity and high cost of its policies.

As a result, the implementation of the two procedures is currently using new methods; some of them do not generally lead to satisfactory results. Thus, to overcome these challenges, the use of the uncertainty profile becomes an asset. The use of this strategy raises a few questions:

- How to choose the most appropriate regression model?
- Can we estimate the two error components: random and systematic by the uncertainty profile?
- How to evaluate the criteria of the analytical validation with our approach?
- Do we have enough arguments to explain all the sources of uncertainty?

Indeed, with the conventional approach, the evaluation of the linearity is done mainly with the use of the lack of fit test. Indeed, in some cases where the variability of the pure error will be significantly less than the fitting error, Fisher's report will be high. Therefore, the fitting error and the pure error will be of a different order.

The linearity of the method will no longer be verified.

Moreover, the separate assessment of accuracy and fidelity was not used to define the quality of future outcomes, but it was used to determine the performance of an analytical method. Its criterion simultaneously represents the systematic and random error. They can be easily characterized in a method validation, when several repetitions are to be planned. However, in routine analysis, a single measurement is performed, so it is impossible to differentiate its two types of errors. Therefore, the validity of the method is related to both the validity of the fidelity and the accuracy.

It is also important to note that with the accuracy profile approach, the use of several response functions is valid; it adopts various functions, whose linearity is not required. Indeed, seven response functions were used, namely: The linear or simple linear model, the linear weighted or quadratic weighted model, and the linear model after a square root transformation and a logarithmic transformation. Therefore, the most appropriate response function is the one that has provided limits of accuracy within acceptance limits. It also goes with the uncertainty profile.

It should be emphasized that both strategies lead to the validity of an analytical method; they can evaluate the difference between a measure (x) and its true value (μ).Indeed, to quantify this difference is to take into account of the total error of measurement (systematic error + random error). On the other hand, the accuracy profile approach presents weaknesses for the estimation of the uncertainty with the β expectation tolerance interval.

To avoid this, the uncertainty profile procedure has been chosen, as a reference for the estimation of uncertainty. While a difference in the estimation of measurement uncertainty was observed, as noted in Table 3,In reality, this difference can be explained by two facts: On the one hand, the total error approach uses the β expectation tolerance interval, On the other hand, the total error approach neglects the term corresponding to the sum of the effects due to the deviation in the uncertainty equation 13 in front of the two main uncertainty components: Type of reproducibility and the standard uncertainty related to the bias of the method.

Therefore, the uncertainty profile approach proves its application value, the results obtained showed a flexibility of the said approach. These results can be generalized in other fields of application. The uncertainty profile therefore suggests a good relationship between analytical validation and uncertainty estimation. According to Saffaj et al, the uncertainty profile overcomes several challenges and examines some additional functions without resorting to complementary experiments comparing to other approaches for estimating uncertainty.

CONCLUSION

In conclusion, the uncertainty profile has been successfully applied for the assay of NVP. It has been proven effective in assessing the performance of the LC-MS method, with a good assessment of the measurement

uncertainty of analytical results. The excellent capacity of Uncertainty profile has been demonstrated. The uncertainty profile has the advantage of estimating the chemical uncertainty without resorting to additional experiments, which conventional validation approaches do not offer.

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