International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

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Estimation of Measurement Uncertainty in Assay of Active Pharmaceutical Ingredient by HPLC Using GUM Approach: Development of Excel Model

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International Journal of Biological and Pharmaceutical Sciences Archive, 2025, 10(02), 121–128

Publication history: Received on 03 August 2025; revised on 07 November 2025; accepted on 10 November 2025

Article DOI: https://doi.org/10.53771/ijbpsa.2025.10.2.0071

Abstract

The evaluation of measurement uncertainty is a major concern in pharmaceutical analysis, particularly for quantitative assays performed using high-performance liquid chromatography (HPLC). This study aims to estimate the uncertainty associated with the assay of an active pharmaceutical ingredient (API) by applying the methodology outlined in the *Guide to the Expression of Uncertainty in Measurement (GUM)*, in accordance with international standards for analytical method validation . HPLC is widely used due to its accuracy, precision, and robustness . Estimating the combined and expanded uncertainty is essential to ensure the reliability of the analytical results . To support the routine application of this approach, an Excel template was developed to perform similar uncertainty calculations in pharmaceutical laboratories.

Keywords: Measurement uncertainty; GUM approach; HPLC; Modelling; Excel template

1 Introduction

The estimation of measurement uncertainty is a fundamental component of modern metrology, particularly in the field of chemical analysis [1]. It is defined as a parameter associated with the result of a measurement that characterizes the dispersion of values that could reasonably be attributed to the measurand [1]. In accordance with ISO/IEC 17025, which requires testing and calibration laboratories to report the uncertainty associated with their analytical results, considering this uncertainty is essential to ensure the reliability, comparability, and traceability of measurements [2]. The Guide to the Expression of Uncertainty in Measurement (GUM), published by the International Bureau of Weights and Measures (BIPM), provides a rigorous and standardized methodology based on a mathematical model of the measurement process, in which the result is a function of several input quantities, each affected by its own uncertainty [1,3]. When applied to the assay of an active pharmaceutical ingredient (API) by high-performance liquid chromatography (HPLC), this approach allows for the identification of key sources of variability—such as uncertainties related to weighing, volume measurements, calibration, and instrumental conditions—and enhances the quality and reliability of analytical results [4]. In the context of pharmaceutical quality control, where the accuracy of assays directly impacts the efficacy and safety of medications, it becomes essential to rigorously estimate measurement uncertainty to ensure compliance with regulatory requirements [2]. This study thus aims to apply the GUM approach to evaluate the measurement uncertainty associated with the assay of an API by HPLC, by identifying the influential input quantities, quantifying their respective uncertainties (type A and type B), and calculating the combined and expanded uncertainty of the final result [1,4].

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2 Materials and Methods

2.1 Materials

2.1.1 Software

The uncertainty estimation calculations were performed using a spreadsheet developed in Microsoft Excel LTSC MSO (version 2403, build 16.0.17330.2000, 64-bit). The spreadsheet incorporates automated formulas and statistical tools to ensure the accuracy and reliability of the analytical computations. [6].

2.1.2 Instrumental and chromatographic conditions [7]

HPLC measurements were carried out on an end-capped C18 column (150 mm × 4.6 mm, 5 µm) with a phosphate/methanol mobile phase (36:65, v/v), a flow rate of 1.3 mL/min, and detection at 272 nm. The injection volume was 10 µL

2.2 Analytical procedure

2.2.1 Preparation of the Standard Solution

« Dissolve a mass 50 of mg (m_{st}) of the active substance SCR in 2,5 ml of methanol R and dilute to 50 ml (V_1) with the mobile phase, dilute 10 ml (V'_1) of the solution to 100 ml (V_2) with the mobile phase » [7]

2.2.2 Preparation of the Test Solution

« Dissolve a mass of 50 mg (m_e) of the substance to be examined in 2.5 ml of methanol R and dilute to 50 ml (V_3) with

2.3 Methodology – Theoretical Part (GUM Approach)

The method proposed by the *Guide to the Expression of Uncertainty in Measurement (GUM)* considers the measurement result as a random variable [1]. The associated uncertainty is thus quantified statistically, and the result must be expressed as a confidence interval [1,3]. The estimation of this uncertainty relies on a rigorous analysis of the measurement process aimed at identifying the main sources of variability, such as random errors, systematic biases, and environmental or instrumental factors [4].

The GUM distinguishes two categories of uncertainty evaluation:

- Type A: based on the statistical analysis of a series of repeated measurements (e.g., repeatability standard deviation) [1,8].
- Type B: based on information from documented sources, such as calibration certificates, instrument specifications, or technical data provided by manufacturers [1,9].

The combination of these uncertainty components is performed using the law of propagation of uncertainties. The final measurement result is then expressed as a central value with its associated expanded uncertainty, providing a confidence level typically of 95% when a coverage factor $k \approx 2$ is used [1,3,9].

It can be represented as:

$$y = \hat{y} \pm U \qquad (1)$$

This approach relies on a mathematical model describing the relationship between the measured quantity and a set of input quantities(x_1, x_2, ..., xn) according to the general formula: [1]

$$y = f(x_1, x_2 \dots \dots . x_n) \quad (2)$$

The combined uncertainty associated with the measurement result is calculated by applying the law of propagation of uncertainties, which uses a first-order Taylor series expansion [1,3]. This method approximates the measurement function as a function of the input quantities. Each individual contribution is weighted by the sensitivity of the output quantity [1,10] relative to that input variable and is calculated by the formula: [1]

$$u_c(Y) = \sqrt{\sum\left(\frac{\partial y}{\partial x_i} \times u(x_i)\right)^2} \quad (3)$$

Where:

- $u_c(Y)$is the combined standard uncertainty of the final result y
- $\frac{\partial f}{\partial x_i}$: is the sensitivity coefficient of y with respect to x_i
- $u(x_i)$: is the standard uncertainty associated with variable x_i

The expanded uncertainty (U), expressing a 95% confidence interval, is then obtained by multiplying the combined uncertainty by the coverage factor k=2

2.4 Cause-and-Effect Diagram (Ishikawa)

An Ishikawa diagram is used to identify all potential sources of uncertainty that may affect the measurement result. These sources are grouped into categories to facilitate analysis [11]:

- Material: Includes the balance, glassware, and chromatographic reproducibility [11].
- Method: Covers the preparation steps and the applied procedure [2].
- Environment: Refers to ambient conditions such as temperature and humidity [12].
- Manpower: Includes uncertainties related to volume reading and operator variability [10].
- Materials: Concerns the reference standard and its purity [4].

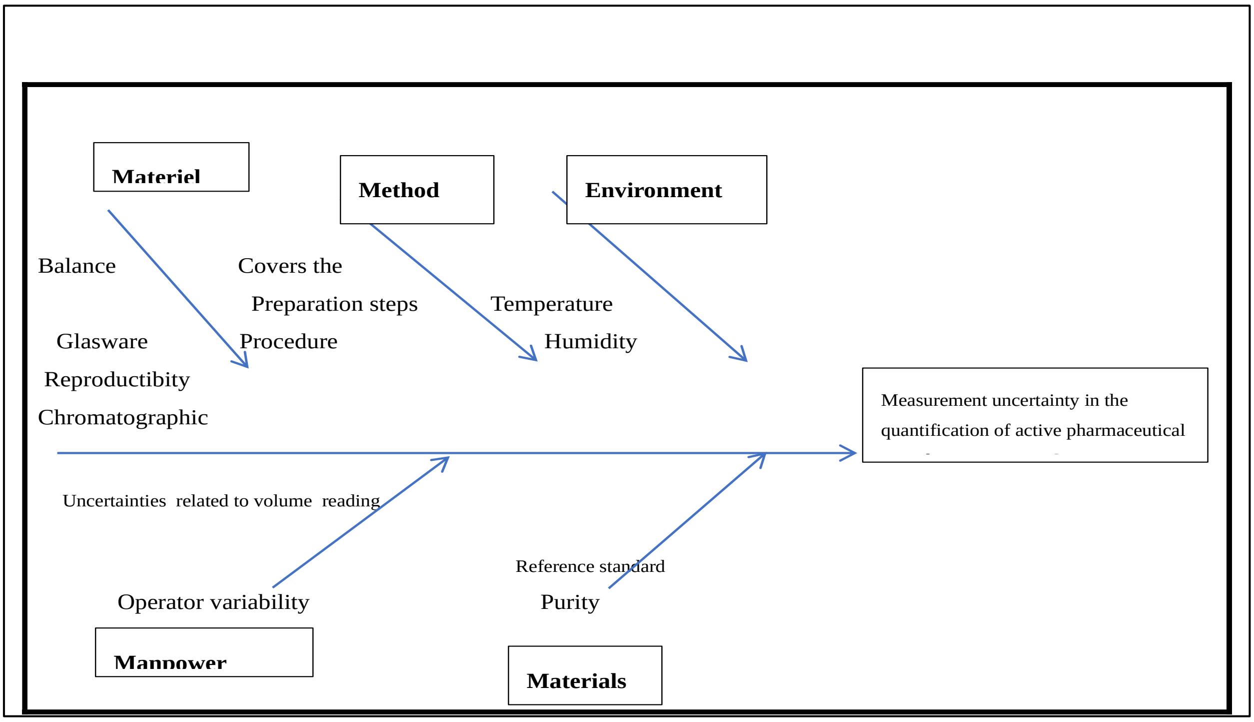

Figure 1 Cause-and-Effect Diagram (Ishikawa)

2.5 Measurement Model – Modelling of the Measurement Process

In the context of High Performance Liquid Chromatography (HPLC), the measurement model used to calculate the quantity y, corresponding to the assay of the active pharmaceutical ingredient (API) in the tested sample, is expressed by the following equation[4,10]:

$$y(\%) = \frac{S_e}{S_{st}} \times \frac{C_{st}}{C_{me}} \times T_{SCR} \times P \quad (4)$$

More explicitly, it may be expanded as [4,10]:

$$y(\%) = \frac{S_e}{S_{st}} \times \frac{m_{st}}{V_1} \times \frac{V_1'}{V_2} \times \frac{V_3}{m_e} \times \frac{V_4}{V_2'} \times T_{SCR} \times P \quad (5)$$

Where the input quantities (random variables) are defined as follows:

- S_e : Peak area of the sample (test solution)
- S_{st}: Peak area of the standard
- $\boldsymbol{m_{st}}$: Mass of the standard substance
- $\boldsymbol{V_1, V_1', V_2, V_3, V_4, V_2'}$ Volumes used at various stages of preparation
- m_e: Mass of the sample

$\boldsymbol{T_{SCR}}$Assay value (purity) of the reference substance, expressed as a percentage

P: Correction factor for water content, derived from T_{H2O}, the water content in the standard

Thus, the mathematical model can be represented as:

Le modèle mathématique

$y = f(S_{st}, S_{me}, m_{st}, m_e, V_1, V_2, V_3, V_4, V_1', V_2', T_{SCR})$ [1,4] (6)

2.6 Variables Contributing to the Measurement Uncertainty of Active Ingredient Assay by HPLC

In order to estimate the overall uncertainty of the assay, it is essential to identify all variables involved in the measurement model and to determine the sources of uncertainty associated with each. For each variable, an evaluation method was selected in accordance with the recommendations of the GUM, distinguishing between Type A evaluations (based on experimental data) and Type B evaluations (based on technical documents, calibration, or analysis certificates)[1].

Table 1 Input variables and uncertainty estimation in HPLC assay

Variable $\boldsymbol{x_i}$	**Source of Uncertainy**	**Estimation Method**
m: masse	Balance calibration certificate	Type B (from calibration certificate values)
V : volume	Uncertainy of pipettes and volumetric flasks	Type B
A : Chromatographic peak area	Repeatability of the HPLC system	Type A (standard deviation from repeated injections)
T_{scr} : Reference substance assay	Certificate of analysis from supplier	Type B (value with associated uncertainty)
T_{H2O} : Water content	Water conent determination (e.g. Karl Fisher method	Type B (certified measurement result)

The table below presents the main input variables of the measurement model used in the assay of an active pharmaceutical ingredient by high-performance liquid chromatography (HPLC). For each variable, the corresponding source of for each variable, the corresponding source of uncertainty is provided.

2.7 Sensitivity coefficients

Sensitivity coefficients are the partial derivatives of the measurement function

$$y = f(x_1, x_2 \ldots\ldots . x_n)$$

with respect to an input variable x_i. They express how a change in an input variable affects the output y. They are mathematically defined as

$$C_i = \frac{\partial y}{\partial x_i} \text{ …….. (7)}$$

To calculate the partial derivatives: $C_i = \frac{\partial y}{\partial x_i}$, the function y can be factored to simplify the derivations [1]. The sensitivity coefficients of the input variables, which are required for the calculation of the combined uncertainty, are summarized in the following table. They represent the variation of the measured quantity as a function of each parameter.

Table 2 Sensitivity coefficients of input variables for combined uncertainty calculation

Variable x_i	**Coefficients de sensibilité $C_i = \frac{\partial y}{\partial x_i}$**
S_{st}	$-\frac{y}{S_{st}}$
S_e	$\frac{y}{S_e}$
$\boldsymbol{m_{st}}$	$\frac{y}{m_{st}}$
$\boldsymbol{m_e}$	$-\frac{y}{m_e}$
$\boldsymbol{V'_1}$	$\frac{y}{V'_1}$
$\boldsymbol{V_2}$	$-\frac{y}{V_2}$
$\boldsymbol{V_3}$	$\frac{y}{V_3}$
$\boldsymbol{V_4}$	$\frac{y}{V_4}$
$\boldsymbol{V'_2}$	$-\frac{y}{V'_2}$
$\boldsymbol{T_{SCR}}$	$\frac{y}{T_{SCR}}$
$\boldsymbol{P}$	$\frac{y}{P}$

2.8 Combined uncertainty

The evaluation of the combined uncertainty is based on the law of propagation of uncertainties, applying a first-order Taylor series expansion of the measurement function around the nominal values of the input quantities

Each sensitivity coefficient $C_i = \frac{\partial y}{\partial x_i}$ reflects the influence of the corresponding input variable x_i on the output y.

The combined uncertainty $u_c(y)$ was then obtained by the square root of the weighted sum of the variances of the input quantities: [1]

$$u_c(y) = \sqrt{\sum_{i=1}^{n}\left(C_i \times u(x_i)\right)^2} \text{ (8)}$$

Where

$u(x_i)$ is the standard uncertainty associated with the input variable x_i

$\boldsymbol{C_i}$ is its sensitivity coefficient.

2.9 Expanded uncertainty

In order to allow the interpretation of the result with a defined confidence level, the combined uncertainty was used to calculate the expanded uncertainty. It was obtained by multiplying the combined uncertainty $u_c(y)$ by a coverage factor k, set to 2 to ensure a confidence level of approximately 95%. The final result of the assay is thus expressed as [1]: $C \pm U$

3 Result

The results obtained from the evaluation of the measurement uncertainty related to the assay of the active ingredient by HPLC are presented in the table below. The combined uncertainty $\boldsymbol{u_c(y)}$ was estimated at [value] %, and the expanded uncertainty U, calculated with a coverage factor k=2, amounts to [value] %, corresponding to a confidence level of approximately 95% [1,3]. The final assay result is thus expressed as: [1]

$$C = \text{measured value} \pm U \quad (9)$$

Table 3 Results of the active substance (API) assay with uncertainty estimation (HPLC)

Trial	**Results (%)**	**Standard Uncertainty**	**Expanded Uncertainty (k=2)**
1	100.18	0.57	1.14
2	100.18	0.57	1.14
3	99.92	0.57	1.13
Average value	100.09	0.32	0.65

Relative Contributions of Uncertainty Sources (Volume and Mass) to the Total Uncertainty

The relative contributions of the uncertainties associated with volume and mass were evaluated based on the standard uncertainties estimated for each source, following the law of propagation of uncertainty. The numerical values used for these calculations, along with the detailed formulas for computing each contribution, are provided in Annex 4 [1,11].

Furthermore, all calculations were automated using a dedicated Excel spreadsheet, ensuring traceability and reproducibility of the results. The table below presents the relative percentages of the volume and mass uncertainties within the total combined uncertainty.

Table 4 Relative Contributions of Uncertainty Sources (Volume and Mass) to the Total Uncertainty

	$\mathbf{u_v}$	$\mathbf{u_m x10^{-4}}$	**% $\mathbf{u_v}$**	**%$\mathbf{u_m}$**
Trial 1	0.52	8.4	91.74	0.15
Trial 2	0.52	8.4	91.72	0.15
Trial 3	0.52	8.4	91.73	0.15
Average value	0.3	4.9	91.73	0.15

The Excel spreadsheet, available via the link below, serves as a template for uncertainty calculation. Simply fill in the unlocked cells to automatically obtain the assay results and the associated uncertainty estimation.

4 Discussion

The results obtained in this study show that the relative combined uncertainty associated with the assay of the active pharmaceutical ingredient (API) by high-performance liquid chromatography (HPLC) is estimated at 0.65 %, with an expanded uncertainty (coverage factor k = 2) corresponding to a confidence level of approximately 95 % [1,3]. This level of precision complies with the requirements of current standards for the quality control of pharmaceutical substances [2,7].

The uncertainty budget analysis reveals that the main source of uncertainty arises from dilution volumes, which contribute approximately 91.73 % to the total uncertainty. This significant contribution is explained by the high sensitivity of the concentration calculation to variations in the measured volumes, highlighting the need for rigorous handling and the use of calibrated and well-maintained measuring equipment.

In comparison, the uncertainty related to the purity of the reference standard is relatively low, contributing around 10 %, reflecting the high quality of the material used[4]. Measurement repeatability also has a meaningful impact, with a contribution of about 3.09 %, emphasizing the importance of performing multiple trials to reduce experimental variability [8].

While the uncertainty associated with volume has a significant impact, that related to mass remains low, with a relative contribution of 0.15 % to the combined uncertainty. This reflects the stability and accuracy of the weighing process [9].

These results are consistent with those reported in the literature, particularly by Smith et al. (2018), who describe a global uncertainty ranging from 0.4 % to 1.2 % for similar assays performed using HPLC [13]. Furthermore, the expanded uncertainty obtained remains within acceptable limits for analytical method validation, thereby supporting the reliability and compliance of the method used [5].

However, some limitations must be considered. The number of trials performed (n = 3) may not be sufficient to fully capture the inherent variability of the process, which could result in an underestimation of the true uncertainty [1]. Additionally, the assumption of independence between the various sources of uncertainty, adopted to simplify the calculations, may not be entirely valid under all experimental conditions [1,11]

To improve the overall accuracy of the assay, several approaches can be considered. The use of high-precision automatic pipettes, regularly calibrated, would significantly reduce the uncertainty related to dilution volumes [2,7]. Furthermore, increasing the number of repeated trials would contribute to a better estimation of repeatability and, consequently, a reduction in the associated standard uncertainty [8].

In conclusion, the methodological approach adopted in this study enables a rigorous estimation of the uncertainty associated with the assay of the active ingredient by HPLC. This estimation ensures the reliability of analytical results and contributes to quality assurance in the context of drug development and control [1,4,5].

5 Conclusion

In summary, this study established a rigorous methodology for estimating the uncertainty associated with the assay of the active pharmaceutical ingredient by high-performance liquid chromatography (HPLC). The adopted approach, compliant with the recommendations of the Guide to the Expression of Uncertainty in Measurement (GUM), incorporates all relevant contributions, notably uncertainties related to dilution volumes, measurement repeatability, and the purity of the standards used

The results demonstrate that the obtained expanded uncertainty ensures sufficient precision for quality control of pharmaceutical substances, in accordance with regulatory requirements Furthermore, the uncertainty budget analysis highlights the main sources of error, thereby providing avenues for improvement to optimize analytical reliability .

This approach can be effectively applied to other analytical assay methods, thus helping to strengthen confidence in the results obtained and to ensure the quality of pharmaceutical products

Compliance with Ethical Standards

Acknowledgments

The authors sincerely thank Mrs. Sonia Sebai Ben Amor, Director of the National Laboratory for Drug Control, for her valuable support and for providing the necessary resources to carry out this work.

Disclosure of Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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[14] Supplementary files related to this manuscript are available online at the following link

[15] https://drive.google.com/file/d/1W9xWfSZURBeP8Ka000FXwI7shufAkzOD/view?usp=sharing