Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of measurement uncertainty of dissolution tests by the ISO-GUM approach and Monte-Carlo simulation

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Abstract

Measurement uncertainty, no doubt, is an important element in examining and interpreting the results obtained in chemical analyses, particularly in the pharmaceutical field. Obviously, this is required by several guides and standards; however, few documents are interested in estimating the measurement uncertainty associated with dissolution test results. In this paper, the Guide to the expression of Uncertainty in Measurement (GUM) and its Supplement 1 "Monte-Carlo Simulation" (MCS) were used, side by side, to estimate the measurement uncertainty of two dissolution test methods, each using a different instrumental technique, namely UV-Visible Spectrophotometry and High Performance Liquid Chromatography (HPLC). A comparative study of both approaches was successfully conducted then with the aim of not only examining the compatibility between the results of the two methods, but also the presentation and discussion of the advantages and limitations of the applicability of each approach in drug analyses. The results obtained with the ISO-GUM and the Monte-Carlo simulation, for the two cases of analysis, revealed comparability between the estimates of uncertainty. Indeed, the standard uncertainties obtained by the two approaches are very close ($u_{GUM\ Repaglinide\ (RG)}$ = 1.1885% & $u_{MCM\ Repaglinide\ (RG)}$ = 1.1854% and $u_{GUM\ Irbesartan\ (IB)}$ = 1.4028 % & $u_{MCM\ Irbesartan\ (IB)}$ = 1.3071%). On the other hand, it has been found that the ISO-GUM approach slightly overestimates the expanded uncertainty because of the used value k = 2 of the coverage factor. Moreover, we have found that the conditions of applicability of the analytical approach and its numerical complement are not always obvious, in particular, when the model of calculation of the measurand is complicated and the instruments of measurements used are complex.

Keywords: Measurement uncertainty; ISO-GUM; Monte-Carlo Simulation; Dissolution test.

1. Introduction

The importance of the measurement results derived from scientific research analyses or industrial activities, is well recognized. In order to use them wisely and to compare them with each other, or with specified reference values or standards of measurement quality, it is requested that these achieved results are reliable and associated with a parameter characterizing their accuracy; that is, the measurement uncertainty.

Thereby, the estimation of measurement uncertainty (MU) has become increasingly necessary in different uses of analysis and testing [1]. Thus, several standards, especially the ISO17025 standard, require that each result obtained is accepted only if it is accompanied by a quantitative affirmation of uncertainty.

In order to adopt standard procedure for estimating uncertainty, the ISO published in 1993 the Guide to the expression of uncertainty in measurement (GUM) with minor corrections and updates in 1995 [2].Then, a EURACHEM document titled "Quantifying Uncertainty in Analytical Measurement", based on GUM principles, was published as a first edition in 1995 to assess measurement uncertainty in the field of analytical chemistry [3].

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Receive Date: 21 September 2020, Revise Date: 31 October 2020, Accept Date: 21 April 2021
DOI: 10.21608/EJCHEM.2021.43264.2882

The principle of the GUM method is based essentially on the law of propagation of variances. This method deals separately with each source of uncertainty found in the measurement process, then the estimates of standard uncertainties of various input quantities are evaluated, combined and associated via the measurement model to finally determine an estimate of the overall standard uncertainty.

Besides the ISO approach [4], a supplement 1to the GUM was published in 2008 by JCGM-101 and in 2012 by EURACHEM/CITAC (3ed) to overcome some of the complexities that the GUM approach is unable to manage [5]. This numerical approach called Monte-Carlo simulation is based on the concept of the propagation of probability distribution functions (PDF) through a combination of different probability distributions of the input parameters by a numerical simulation to obtain the PDF of the measurand [6-8].

Moreover, several types of analysis are carried out in pharmaceutical laboratories. Their results associated with measurement uncertainties are indicators of the quality of drugs. Although several normative documents requiring the evaluation of measurement uncertainty are evolved, the calculation of MU is still a practical problem in pharmaceutical laboratories**.** In this regard, given the high importance of the subject, the journal STP PHARMA recently published a document entitled "Measurement uncertainty of the analytical methods in drug monitoring" [9].

In addition, one of the essential drug analyses is the dissolution test, which attracts not only the attention of researchers and pharmaceutical industry players, but also regulatory authorities. Thus, this test obviously contributes to the evaluation of the biopharmaceutical quality of a product at different stages of its life cycle. Indeed, the in vitro dissolution allows to appreciate the changes in the production site, the manufacturing process or the development of the formulation and the quality control which is translated by a quality test from batch to batch before the product reaches the market. The dissolution test is also considered as a necessary criterion helping to make decisions about the studies of bioavailability and bioequivalence. In other words, the drug must be solubilized in the aqueous medium of the gastrointestinal system to be absorbed.

On the other hand, despite the important position occupied by the dissolution test method in the pharmaceutical field and the critical results they produce, few are the published documents dedicated to the evaluation of the measurement uncertainty associated with the result of the dissolution test. In addition, none of these documents evaluated the effect and contribution of the critical parameters of the dissolution test of solid oral dosage forms to the overall budget of measurement uncertainty.

This paper aims to propose and illustrate a practical approach to calculate the measurement uncertainty of the dissolution test. Indeed, this present study has multiple objectives. First, we want through this work to contribute to the orientation of the pharmaceutical laboratories to a good estimation of measurement uncertainty using the analytical approach ISO-GUM and numeric approach by the simulation of Monte-Carlo. As a result, this contribution is accomplished by describing and reporting step by step the uncertainty estimation techniques through the study of two dissolution test cases, each using a different instrumental technique for the dosage namely the spectrophotometry in UV-Visible and liquid chromatography (HPLC). At the same time, we also aim to present a comparison of the uncertainty estimates obtained by the two approaches to examine the compatibility of the uncertainties results. Finally, based on the steps followed by the assessment of uncertainty and the results obtained, we will present a critical discussion to highlight the conditions and the limits of application of these two approaches in the field of drug control in the pharmaceutical laboratories.

2. Materials and methods

2.1. Case Study # 1: Dissolution test of Repaglinide-HPLC assay

2.1.1. Dissolution conditions of the solid oral form

The dissolution tests were carried out in a Dissolutest Sotax, model AT7 Smart. The apparatus is equipped with six containers partially immersed in a thermostated water bath and equipped with a manual sampling system with graduated of syringes 10 mL. The dissolution test was carried out with a rotation speed of 50 rpm in a dissolution medium consisting of 1000 mL of a 0.1N hydrochloric acid solution. After preparing the water bath at (37 ± 0.2°C), one tablet was placed in each of six containers containing the specified volume of dissolution medium. After 30 minutes of rotation, 10 mL samples were taken from each Repaglinide dissolution container and then

filtered through a 0.45 µm PTFE syringe filter, then a 5 mL dilution of the filtrate at 10 mL was made with the dissolution medium.

2.1.2. Chromatographic conditions

The High performance liquid chromatography was performed using a WATERS "ALLIANCE" type chromatograph, which is characterized by a Waters 2489 dual λ double-wavelength UV / visible detector, an Empower 2 software. The analytical column is BDS hypersil C18 250 mm 4.6 mm 5µm. The separation is carried out isocratically with a flow rate of 1.5 mL / min and the temperature of the column is 45 ° C.

The mobile phase consists of a filtered and degassed mixture of pH 3.2 buffer solution (4 g of KH_2PO_4 in 1000 mL of purified water adjusted with orthophosphoric acid) and acetonitrile (30:70; V / V). The injection volume of the sample is 100 µL. The detection is carried out at a wavelength of 245 nm.

2.1.3. Preparation of the Repaglinide STANDARD solution

In a 100 mL volumetric flask, 10 mg of Repaglinide (RG) was dissolved in the mobile phase (concentration 0.1 mg mL^{-1}). Then a dilution of 5mL of this solution to 100 mL with the same solvent was made. Then a second dilution of 5 mL of this solution in 50 mL was carried out with the dissolution medium to finally have a concentration of 0.0005 mg mL^{-1}.

2.2. Case Study # 2: Dissolution test of Irbesartan-assay by UV/VIS spectrometry

2.2.1. Conditions of dissolution

The dissolution test was carried out using the Sotax dissolution apparatus, the rotational speed was set at 50 rpm. The dissolution medium used is a solution of 1000 mL of the 0.1N hydrochloric acid solution. After heating the water bath to a temperature of (37 ± 0.5°C), one tablet was placed in each of the six containers containing the dissolution medium. After 45 minutes of rotation, 10 mL aliquots were taken from each container and then filtered through a 0.45 µm PTFE syringe filter. Then, a 2 mL dilution of the filtrate at 25 mL with the dissolving medium was performed.

2.2.2. Spectrophotometric equipment

A UV-visible spectrophotometer (Perkin Elmer, Lambda 25), operating in the range of 190-1100 nm was used to measure the absorbances of Irbesartan test solutions.

The absorbance of the standard solution and the solutions to be examined were determined at 254 nm using the dissolution medium as the blank solution.

2.2.3. Preparation of the Irbesartan solution

In a 100 mL volumetric flask, 60 mg of Irbesartan (IB) was dissolved in 25 mL of methanol. After complete dissolution in ultrasound, a volume dilution was made with the dissolution medium, then a second dilution (2 mL in a 100 mL flask) was carried out with the same solvent.

2.3. Approaches to Assessing Measurement uncertainty

2.3.1. Calculation principles by ISO-GUM approach

The guide to the expression of uncertainty in measurement (GUM) is currently available in several publications such as ISO / IEC guide 98-3: 2008 [10, 11], NIST TN 1297[12] , and EA-4/02 to establish a standard procedure based on consistent rules for the evaluation of measurement uncertainty [13].

EURACHEM/CITAC "Quantifying the Uncertainty of Analytical Measurement" is the most famous guide that adopted the GUM in the early 1990s in the field of analytical chemistry, which aims to demonstrate how the notions of GUM can be applied to analytical chemistry.

The analytical approach (GUM) estimates the overall measurement uncertainty by identifying, quantifying and combining all the sources of uncertainty associated with the measurand Y[14], which is most often determined from n input quantities $X_1, X_2 .. X_n$through the following functional relationship f.

$$Y = f(X_1, X_2, .., X_n) \quad (1)$$

Where $X_1, X_2, .., X_n$ are parameters having an influence on the measurand Y (Fig.1).

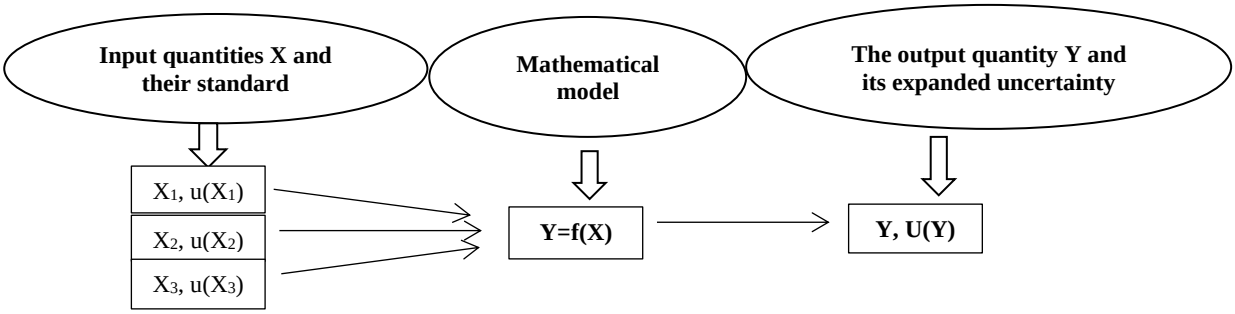

Fig. 1. Principle of the GUM meth

The estimate of the measurand Y, denoted y, is obtained by substituting the input estimates $x_1, x_2..x_n$ for the values of n quantities $X_1, X_2..X_n$ in equation (1). Thus the estimate y, which is the result of the measurement, is given by:

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

A first-order Taylor series approximation of $Y = f(X_1, X_2..X_n)$ for a small deviation of y about Y in terms of small deviations of $x_i$about X_i is expressed by:

$$(y - Y) = \sum_{i=1}^{n} \frac{\partial f}{\partial x_i}(x_i - X_i) \qquad (3)$$

Where all higher order terms are assumed to be negligible. The square of the deviation $y - Y$ is then given by:

$$(y - Y)^2 = [\sum_{i=1}^{n} \frac{\partial f}{\partial x_i}(x_i - X_i)]^2 \qquad (4)$$

Which we can write again in the developed form:

$$(y - Y)^2 = \sum_{i=1}^{n} \left[\frac{\partial f}{\partial x_i}\right]^2 (x_i - x_i)^2 +$$
$$2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \frac{\partial f}{\partial x_i} \frac{\partial f}{\partial x_j}(x_i - X_i)(x_j - X_j) \qquad (5)$$

From equation (5), we deduce the variance of y:

$$u^2(y) = \sum_{i=1}^{n} \left[\frac{\partial f}{\partial x_i}\right]^2 u^2(x_i) +$$
$$2 \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \frac{\partial f}{\partial x_i} \frac{\partial f}{\partial x_j} u(x_i, x_j)\rho_{ij} \qquad (6)$$

With:

$u(y)$: The standard uncertainty of the estimate y obtained by combining the standard uncertainties of the input estimates $x_1, x_2..x_n$, denoted $u(x_1)$, $u(x_2)$ … $u(x_n)$.

$\frac{\partial f}{\partial x_i}$: The partial derivative or the sensitivity coefficient linking the change in the measurement result y to the input estimates x_i .

ρ_{ij} : The correlation coefficient between x_i and x_j

$u(x_i, x_j)$: The covariance of x_i, x_j.

In the case where the input quantities X_i are independent or uncorrelated, equation (6) is expressed as follows:

$$u^2(y) = \sum_{i=1}^{N} \left[\frac{\partial f}{\partial x_i}\right]^2 u^2(x_i) \qquad (7)$$

By posing: $C_i = \frac{\partial f}{\partial x_i}$

Then, $u^2(y)$ is written as the sum of the squares of the standard uncertainties weighted by their squared sensitivity coefficient.

We obtain: $u^2(y) = \sum_{i=1}^{N} C_i{}^2 u^2(x_i)$ (8)

Equation (7) can also be written (if the model contains products or quotients of independent input quantities) in the following form:

$$\frac{u^2(y)}{y^2} = \frac{u^2(x_1)}{x_1^2} + \frac{u^2(x_2)}{x_1^2} + \cdots + \frac{u^2(x_n)}{x_n^2} \qquad (9)$$

Furthermore, two ways to evaluate the standard uncertainties are usable according to the nature of the sources of uncertainty; a type A assessment of the standard uncertainty is carried out by a statistical analysis of series of observations and a Type B assessment of standard uncertainty which is based on scientific judgment, using all available information about the possible variability of the input quantity such as the manufacturer's specifications, data provided by calibration certificates, other certificates, experimental data, etc [15, 16].

The expanded uncertainty expresses an interval around the measurement result. This interval includes a large fraction of the distribution of values that could be attributed to the measurand. It is obtained by multiplying the combined standard uncertainty u (y) by a coverage factor k according to the level of confidence required. For a normal distribution, k = 2 corresponds to an approximate confidence level of 95% and k = 3 to 99.7% [17].

In addition, concerning nonlinear mathematical functions, high order Taylor numerical approximation methods may be required. To overcome these difficulties of mathematical calculation, another alternative approach for estimating measurement

uncertainty can also be used such as Monte-Carlo simulation (MCS) [18].

2.3.2. Principle of the numerical approach

The Monte-Carlo simulation (MCS) approach is a supplement to the guide to the expression of uncertainty in measurement "GUM" published in 2008 by the Joint Committee for Guides in Metrology (JCGM) [10]; it aims to bring added value to the GUM by clarifying new concepts for the evaluation of (MU), which are not explicitly addressed in the GUM method.

Monte-Carlo simulation is an alternative method for estimating MU based on the principle of propagation of distributions rather than the propagation of uncertainties used in the GUM approach [19].

As illustrated in Figure 2, the principle of this numerical approach appears clearly in the fact that the calculation model used describes the measurement process according to the nature of the law of probability of the individual factors affecting the measurand. Indeed, it allows to obtain a statistical distribution of the output variable Y, by simulating each input data according to the corresponding distribution laws (normal, triangular, rectangular, and other laws).

The method of evaluation of uncertainty by propagation of distributions can be summarized in the following steps [10]:

- Analyze the measurement process in order to define the measurand and the influencing factors; the Ishikawa diagram is a practical way to synthesize this analysis whether for the ISO-GUM method or the MCS method.
- Associate with each input variable a random variable governed by the most appropriate probability law (Normal law, rectangular law, joint distribution in the case of correlated variables, etc.); this choice of distribution is based on the information available on the input quantity.
- Choose the number of simulations, denoted M, for the input quantities by drawing in their probability density function (PDF) to deduce the corresponding values for the output quantity, which requires having a pseudo-random number generator sufficiently perform [20]. The supplement 1 to the GUM recommends a value of $M = 10^6$ of simulations so as to ensure that the empirical distribution of the output quantity is sufficiently stable.
- Compute via the mathematical model the M values obtained of the output quantity, which makes it possible to construct the empirical distribution of the measurand.
- Synthesize the results obtained on the measurand which are the mathematical expectations, the standard deviation and the confidence interval, for a given probability level (often 95%).

This alternative approach has several advantages compared to the approach based on the law of propagation of uncertainty [7]:

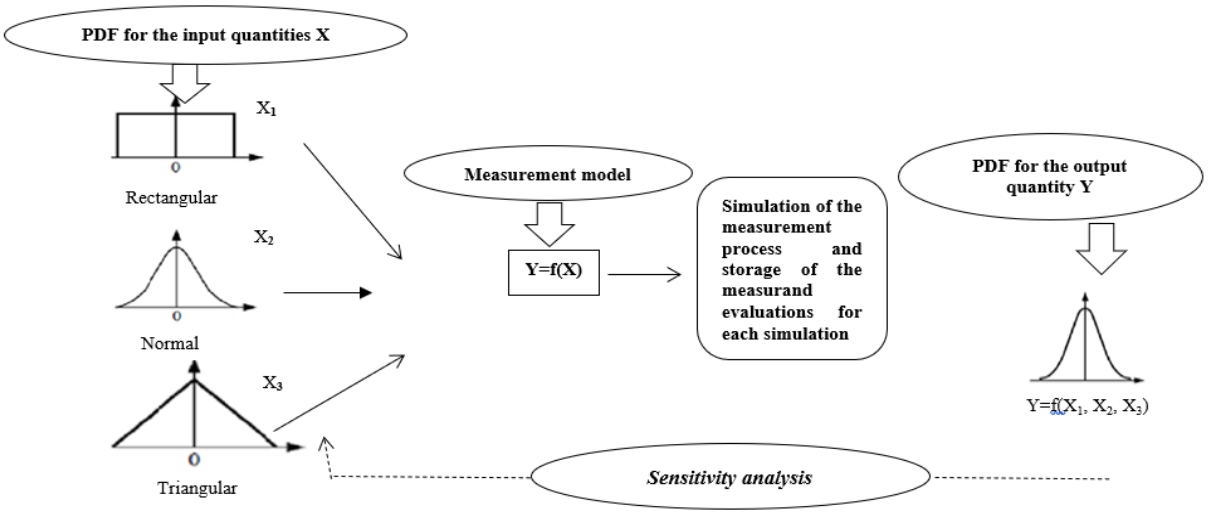

Fig.2. Principle of the Monte -Carlo method

- It is not necessary to calculate partial derivatives as well as the contribution sensitivity coefficients of each influence factor on the measurand.
- It is not necessary to calculate the effective degrees of freedom.
- It can treat any specification function even if it is non-linear.
- It can treat input quantities that are not independent.
- It can work with any probability density function of the input quantities.

This approach conforms with the general principles of GUM; however, the Monte-Carlo simulation still has limitations, particularly because of the necessary computing power that can be very important in the case of a complex system with a large number of input quantities.

2.4. Validation of the results obtained by the ISO-GUM approach.h using a Monte-*Carlo* method

Monte-Carlo simulation can be used to validate the results of the uncertainty propagation law by comparing the extended intervals obtained by the two methods. Indeed, the validation procedure described in the Supplement 1 to the GUM consists in verifying if the confidence intervals obtained by the GUM approach and those obtained by the MCS approach are in agreement for a numerical tolerance close to $\delta = 0.5 \times 10^{l}$ calculated by expressing the standard uncertainty obtained by GUM as follows:

$$u(y) = c.10^{l} \qquad (10)$$

With: l is an integer, c is also an integer with n_{dig} digits (n_{dig} = 1 or 2 depending on whether we validate the GUM method with 1 or 2 significant digits).

The next step is to compare the expanded uncertainties obtained by each method by establishing the absolute differences d_{low} and d_{high} below:

$$d_{low} = |L_{low} - y_{low}| \text{ and } d_{high} = \left|L_{high} - y_{high}\right| \qquad (11)$$

With:

$L_{low} = y - U_{P\%}$ and $L_{high} = y + U_{P\%}$ are the two limits of the interval obtained by the GUM method.

$U_{P\%} = k.u(y)$ is the expanded uncertainty for a confidence level (CL) P% obtained by the GUM method. And k is a coverage factor that corresponds to the CL P%.

y_{low} and y_{high} are the bounds of the extended interval obtained by the Monte-Carlo simulations.

Decision rule: If the two differences of d_{low} and d_{high} are both less than the degree of approximation δ, then the results obtained by the GUM method are comparable to the results obtained by the MCS method. The assumptions of the GUM method are then approved. If this is not the case, MCS or another appropriate approach should be used instead of the uncertainty propagation method such as the total error approach.

2.5. *Evaluation* of measurement uncertainty using the GUM approach

2.5.1. Dissolution test of Repaglinide

The objectives of this part of the study are to identify the principal sources of uncertainty, to quantify the standard deviation of each source of uncertainty, and to calculate the combined and expanded uncertainties for the HPLC method for determining the content of Repaglinide (RG) after 30 minutes of dissolution of the tablet in the dissolution medium.

Step 1: Specification of the measurand

The concentration of Repaglinide found is calculated by calibration with a single point (value 100%); thus the mathematical model for finally calculating the RG content in a tablet after a dissolution test is given by equation 12:

$$T(\%) = \frac{A_s}{A_{st}} \times \frac{W_{st}}{dose} \times P \times D \times 100 \qquad (12)$$

With:

A_s and A_{st} are respectively the (RG) areas of the test solution and the standard solution,

W_{st}: weight of the reference substance of (RG) in mg, P: Purity of the reference substance of (*RG*),

D: is the effect of the dilution factor during the preparation of the test solutions and standard solutions.

Dose: Theoretical content equal to 1 mg.

Step 2: Analysis and quantification of sources of uncertainty

The purpose of this step is, on the one hand, to identify and analyze all pertinent sources of uncertainty using a cause-and-effect diagram known as the Ishikawa diagram, and on the other hand the different contributions to the measurement uncertainty

of the Repaglinide content will be quantified either directly using the experimental results (type A) or deduced from a type B analysis.

In the context of the GUM approach, the Ishikawa diagram is considered as an effective means to prevent the risk of neglecting some sources or being counted twice. Such a scheme represents the causes and effects of the sources of uncertainty for both the dissolution procedure and the HPLC assay method (Figure 3).

In addition, the mathematical formula used to express the measurand calculation result does not always describe the complete analytical procedure exhaustively, because many sources with a strong influence on uncertainty are not often taken into account, whereupon it is necessary to make some modifications on the equation so as to take into account these factors in the final budget of the calculation of the uncertainty. Therefore, to adjust the additional quantities that may have an influence, the (RG) content calculation equation is developed by introducing correction factors that are also indicated in the Ishikawa diagram. Thus, the final model obtained is given by equation 13:

$$T(\%) = \frac{A_s}{A_{st}} \times \frac{W_{st}}{dose} \times P \times D \times R \times F_{DS} \times 100 \quad (13)$$

With:

F_{DS}: Factor of the dissolution system, it includes the effect of the dissolution temperature, the effect of the speed of rotation and also the dissolution time.

R: is the factor of the precision of the method.

According to the Ishikawa diagram, the sources of uncertainty for each parameter affecting the measurement of (RG) content are:

Precision (R)

The precision of the method is determined by performing all the steps of the operating mode of the method. Therefore, it is useless to consider all the contributions to the repeatability separately from each source studied, they are grouped into a single contribution (see diagram). The value of the repeatability is calculated by carrying out three series of six independent repetitions, it is:

$$S_R = S_r/\sqrt{n} \quad (14)$$

And $$RSD_R = S_r/(\bar{A} \times \sqrt{n}) \quad (15)$$

With:

$\bar{A}$: is the average of peak areas.

The standard deviation of the precision and the relative precision are therefore 0.00182 and 0.0021 respectively.

Peak areas (A_s, A_{st})

The concentration of Repaglinide directly depends on the integrated peak areas. The surface itself is affected by different sources of uncertainty. ***Vicki and Barwick*** discussed the main sources of variation affecting a liquid chromatographic analysis. Indeed, these authors have shown, in their review, that chromatographic conditions especially the composition of the mobile phase, the flow rate, the column temperature, the detection by UV spectrophotometry and the injection system are factors that cause the variability of retention time and integration of the peak area. The evaluation of the effect of these factors is accomplished by calculating the precision of the HPLC system (area and retention time) during the system compliance test.

The repeatability of the integration of the peak area and of the retention time is determined from the HPLC system compliance test by performing a series of six injections of the standard solution of RG. The Table 1 summarizes the calculation of standard deviations and CV % of retention time and integrated area.

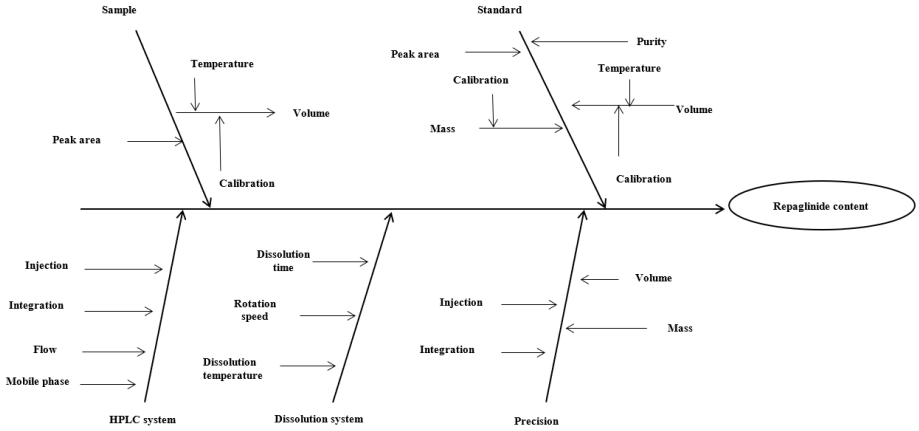

Fig. 3: Ishikawa diagram showing the causes and effects including sources of uncertainty for the HPLC dissolution analysis procedure

Table 1: Calculation results of standard deviation and CV% of area and retention time of the repaglinide dissolution test.

	Mean	CV (%)	Standard deviation, S	Standard deviation of the mean, $S/\sqrt{n}$	u_{HPLC}
Retention time	4.895	0.08	0.003817	0.00156	0.00167
Area	0.973	0.15	0.001472	0.0006	

u_{HPLC}: Standard uncertainty of HPLC system

The standard uncertainty due to the effect of the chromatographic measurement system is given by the following calculation:

$$u_{HPLC} = \sqrt{S_{RT}^2 + S_{Area}^2} = \sqrt{0.00156^2 + 0.00060^2}$$
$$u_{HPLC} = 0.00167 \qquad (16)$$

Dilution (D)

The volume of the solutions contained in the volumetric flasks or delivered with the pipettes are subject to three sources of uncertainty, a0s all volumetric measuring devices, which are the variability or the repeatability of the delivered volume, the uncertainty on the internal volume of the material, and the temperature variation of the test solutions (difference compared to the calibration temperature of the material 20 ° C).

Calibration: Using the uncertainty indicated in the manufacturer's catalog of the instruments used, the standard uncertainty of calibration is calculated assuming the distribution is triangular.

Temperature: According to the manufacturer, the equipment has been calibrated at a temperature of 20 ° C, while the laboratory temperature varies between ± 4 ° C. The resulting uncertainty of this effect can be calculated from the variation of the temperature according to the coefficient of expansion of the liquid. Only the coefficient of expansion of water volume is considered and equal to 2.1×10^{-4} °C^{-1} . The standard uncertainty of the temperature variation is calculated considering that the distribution is rectangular.

Table 2 summarizes all calculations of standard uncertainty of calibration and temperature taking into account the number of times that each instrument is used for dilution, thus the relative standard uncertainty of dilution is found equal to 0. 00349.

Tablet dissolution system (F_{DS})

The good conformity of a dissolution test apparatus depends on four critical parameters namely: the temperature of the dissolution medium, the test time, the rotation speed and the volume of the medium. The effects of these sources of uncertainty are included in the cause and effect diagram presented in Figure 3.

Volume of the dissolution medium

The instrument used to measure the volume of the dissolution medium is a measuring cylinder of 1000 mL; its contribution is already taken into account in the standard uncertainty due to the dilution factors.

Dissolution temperature

The variation of the temperature of the dissolution medium does not exceed ± 0.2 ° C. It should be noted here that the containers must be well closed by the covers to prevent the evaporation of the liquid and also the heat transfer with the air. The average temperature during the dissolution test is 36.5°C. It is assumed that the distribution is triangular, so the standard uncertainty of the temperature effect is:

$$u_{DT} = \frac{(0.2/36.5)}{\sqrt{6}} = 0.002237°C$$

Table 2: Calculation of the relative un0certainty of dilutions of the Repaglinide dissolution test.

0	Volume (mL)	Number of times used	Calibration	Standard uncertainty of calibration	Temperature effect	Standard uncertainty of temperature	uncertainty of volume	Relative uncertainty of volume	(relative uncertainty)²	Relative uncertainty of dilution
Pipet0te	5	3	0.015	0.00612	0.0042	0,00242	0.01141	0.0023	0.000005	**0.00349**
Flask	10	1	0.025	0.01021	0.0084	0,00485	0.01130	0.0011	0.000001	
Flask	50	1	0.06	0.02449	0.0420	0,02425	0.03447	0.0007	0.0000005	
Flask	100	2	0.1	0.04082	0.0840	0,04850	0.08965	0.0009	0.000001	

measuring cylinder	1000	1	5	2.04124	0.8400	0,48497	2.09806	0.0021	0.000004

Rotation speed

According to the study of D.C.Romero et al [21]. The rotation speed is a critical parameter of dissolution; its effect is very significant. The speed of rotation of the test is 50 ± 1 rpm; it is assumed that the distribution is rectangular then the standard uncertainty is:

$$u_{RS} = \frac{(1/50)}{\sqrt{3}} = 0.01155\ tr.min^{-1}$$

Dissolution time

The stopwatch has a precision of 0.01s = 0.0002 min, and since we have no information on the confidence level nor on the nature of distribution, it is assumed that the time grandeur follows a triangular distribution. The standard uncertainty of time, which also depends on the dissolution time, is:

$$u_{Dt} = \frac{(0.0002/_{30})}{\sqrt{6}} = 0.000002\ min$$

Finally, the standard uncertainty due to the factors of the dissolution system is calculated by combining the different standard uncertainties:

$$u_{SD} = \sqrt{u_{DT}^2 + u_{RS}^2 + u_{Dt}^2} = \sqrt{0.002237^2 + 0.01155^2 + 0.000002^2} = 0.01176 \quad (17)$$

The F_{SD} factor, which takes a value of 1, has a relative standard uncertainty RSD_{SD}= 0.01176.

Mass of the standard (W_{st})

The mass of the active ingredient of Repaglinide is determined by a tared weighing giving a mass equal to 10.02 mg. The manufacturer's literature identifies three uncertainty sources for the tared weighing: the repeatability and the contribution due to the error of the calibration function of the scale. This calibration function has two potential sources of uncertainty identified as the sensitivity of the balance and its linearity. The sensitivity can be neglected since the mass obtained by difference is done on the same balance over a very narrow range.

Linearity: the balance calibration certificate indicates an uncertainty of ± 0.02 mg for the linearity; the supplier recommends using a rectangular distribution to convert the linearity contribution to a standard uncertainty.

The contribution of the linearity of the balance is conforms to: $u_{lin} = \frac{0.02}{\sqrt{3}} = 0.0115\ mg$

This contribution has to be counted twice, once for the tare and another for the global weighing, since the effects of linearity are not correlated.

Then: $u(W_{st}) = \sqrt{2 \times 0.0115^2} = 0.0163 mg$

Purity (P)

The purity of the (RG) standard is indicated in the supplier's certificate as being equal to 99.99% ± 0.01%, P is therefore equal to 0.9999 ± 0.0001. It is assumed that the distribution is rectangular since no additional information is available:

$$u(P) = \frac{0.0001}{\sqrt{3}} = 0.00006$$

The contribution of different sources of uncertainty (in terms of relative uncertainties) to the combined standard uncertainty of (RG) content is presented in Figure 4. It is obvious that the contribution of the uncertainty of the dissolution system factor is the most important, followed by the contribution of the dilution factor.

The repeatability, the sample area, the standard area and the mass present an uncertainty of the same order of magnitude, while the uncertainty of purity has practically no influence on the overall uncertainty.

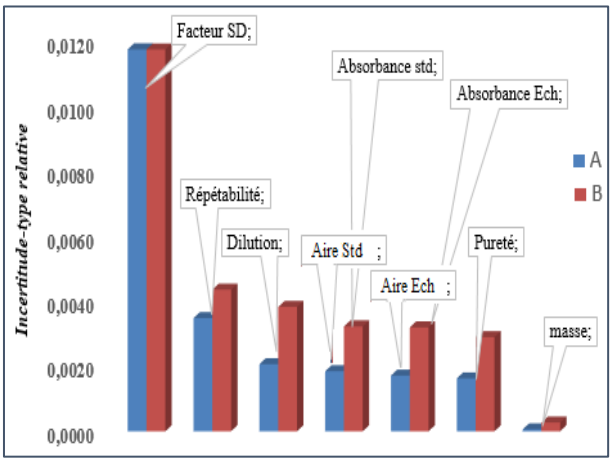

Fig.4. Contribution of different sources of uncertainty in the estimated uncertainty of Repaglinide content (A) and Irbesartan content (B)

Step 3: Calculation of the combined standard uncertainty

In this 3th step, the standard uncertainties of all sources, identified by the Ishikawa diagram, are combined according to the equation of the formula 9 to give the combined standard uncertainty of the content (T%):

$$u_{(T)} = T\sqrt{\frac{u^2(A_S)}{A_S^2} + \frac{u^2(A_{St})}{A_{St}^2} + \frac{u^2(W_{St})}{W_{St}^2} + \frac{u^2(P)}{P^2} + RSD^2{}_D + RSD^2{}_{DS} + RSD^2{}_R} \quad (18)$$

T is the Repaglinide content calculated by equation (13):

$$T(\%) = \frac{0.902}{0.973} \times \frac{10.02}{1} \times 0.9999 \times \frac{1}{10} \times 1 \times 100$$

$$T(\%) = 92.863\%$$

RSD_D: is the relative standard uncertainty of the dilution factor;

RSD_{DS}: is the relative standard uncertainty of the dissolution system factor;

RSD_R: is the relative standard uncertainty of the repeatability;

$$u_{(T)} = 1.19\%$$

The expanded uncertainty $U_{95\%}$ is calculated by multiplying the combined standard uncertainty by a coverage factor equal to 2.

$$U(T) = 1.19 \times 2 = 2.38\ \%$$

The expression of the final result of the Repaglinide content and its estimated uncertainty, according to GUM and Eurachem while respecting the rounding rules, is given as follows:

$$T(\%) = (92{,}9 \pm 2{,}4)\%$$

Dissolution test of Irbesartan

This part of the study aims to estimate the measurement uncertainty, by the GUM method, of the content of Irbesartan (IB) containing in tablets during the dissolution test. The Determination of the (IB) (measurand) content in the test solution was performed by UV/VIS spectrophotometry at 254 nm.

In order to unambiguously identify the different sources of uncertainty of the dissolution test associated with the UV/VIS spectrometric assay, a cause and effect diagram has been established (Figure5).

The equation for calculating the content of (IB) in the test solution taking into account all the factors including those of dilution, dissolution and repeatability is given by the following formula:

$$T\% = \frac{DO_S}{DO_{St}} \times \frac{W_{St}}{dose} \times D \times P \times R \times F_{DS} \times 100 \quad (19)$$

With:

DO_s: is the optical density of the test solution.

DO_{st}: is the optical density of the standard solution.

W_{st}: is the weight of the reference substance of Irbesartan in mg.

Dose: is the theoretical dose of active ingredient in the tablet that is equal to 150 mg.

The calculation results of standard uncertainty of calibration and temperature of dilution volumes are summarized in Table 3 and the relative uncertainty of dilution is found to 0.00383.

Absorbance measurement

The uncertainty associated with the absorbance measurement was estimated from the specifications of the Lambda spectrometer used in the assay method. Indeed, these specifications indicate a typical value of 0.02% for stray light, peak-to-peak background noise (baseline) below 0.0003, stability below 0.0003 per hour, and linearity of the baseline equal to ± 0.001. Their standard uncertainties were calculated assuming a rectangular distribution (Table 4). On the other hand, the Equipment Logbook specifies an uncertainty of ± 0.003 for photometric accuracy, its standard uncertainty is calculated assuming a triangular distribution. Finally, the standard uncertainty due to the absorbance measure calculated by combining the 5 sources of uncertainty is 0.00136. The overall calculation is summarized in Table 4.

Precision (F_{rep})

The value of the repeatability is also calculated in the same way by performing three sets of six independent repeats, then:

$$S_P = S_r/\sqrt{n} \quad (14)$$

And $$RSD_p = S_r/(\bar{x} \times \sqrt{n}) \quad (15)$$

With

$\bar{x}$ is the average of the absorbances.

The standard deviation of the precision and the relative accuracy are therefore 0.00186 and 0.00436 respectively.

Dissolution system (F_{DS})

The parameters constituting the sources of uncertainty of the dissolution system are the temperature of the dissolution medium, the time of dissolution, the rotation speed, and the volume of the medium. Their contributions to uncertainty are calculated in the same way as in the Repaglinide dissolution test. The standard uncertainty of the dissolution system is obtained by combining the uncertainties of the 3 parameters:

$$u_{SD} = \sqrt{u_{DT}^2 + u_{RS}^2 + u_{Dt}^2} = \sqrt{0.000002^2 + 0.011547^2 + 0.002219^2} = 0.011578 \quad (17)$$

Table 5 summarizes the details of calculations.

Table 3: Calculation of the relative uncertainty of dilutions of the Irbesartan dissolution test.

Instrument	Volume (mL)	Number of times used	Calibration	Standard uncertainty of calibration	Temperature effect	Standard uncertainty of temperature	uncertainty of volume	Relative uncertainty of volume	(relative uncertainty)²	Relative uncertainty of dilution
Pipette	2	2	0.01	0.004082	0.00168	0.00097	0.00593	0.0030	0.000009	0.003830
Flask	25	1	0.04	0.01633	0.021	0.01212	0.02034	0.0008	0.000001	
Flask	100	2	0.1	0.04082	0.084	0.04850	0.08965	0.0009	0.000001	
measuring cylinder	1000	1	5	2.04124	0.84	0.48497	2.09806	0.0021	0.000004	

Table 4: Calculation of the standard uncertainty of the absorbance in the Irbesartan dissolution test.

Item	Specification	Extended (2a)	Half-extended, a	Standard uncertainty	Standard uncertainty of absorbance
Stray light	0.02%	0.0002	0.0001	0.000058	0.00136
Linearity of baseline	±0.001	0.002	0.001	0.000577	
peak to peak background noise	0.0003	0.0003	0.00015	0.000087	
Drift	0.0003/h	0.0003	0.00015	0.000087	
Photometric accuracy	±0.003	0.006	0.003	0.001225	

Table 5: Calculation of standard uncertainty of dissolution system in the Irbesartan dissolution test.

Item	Mean value	Specified uncertainty	Half-extended, a	Standard uncertainty	Uncertainty of dissolution system
Rotation speed, tr.min^{-1}	50	±1	1	0.011547	0.011758
Dissolution time, min	45	±0.01 s	0.00017	0.000002	
Dissolution temperature, °C	36.8	±0.2°C	0.2	0.002219	

Mass (W_{st}) and purity (P)

The standard uncertainty of the mass is calculated by counting twice the contribution of the linearity; its absolute and relative value is 0.01633 and 0.00027 respectively.

The purity of IB is noted in the supplier's certificate as being equal to 0.995 ± 0.005. Assuming a rectangular distribution, the standard uncertainty is found equal to 0.00289.

In this application, the contributions of different parameters are indicated in Figure 4, also in this case the contribution of the dissolution system factor which contributes the most to the combined uncertainty followed by the repeatability.

The dilution factor, purity, the absorbance of the standard, and the sample have a relatively similar uncertainty, while the mass is still of a lower order of magnitude.

Combined and expended uncertainty

Using the variance propagation equation, the combined standard uncertainty is calculated by combining the uncertainty contributions of the input parameters and the factors given in the model of equation 18:

$$u_{(T)} = T\sqrt{\frac{u^2(DO_S)}{{DO_S}^2} + \frac{u^2(DO_{St})}{{DO_{St}}^2} + \frac{u^2(W_{St})}{W_{St}^2} + \frac{u^2(P)}{P^2} + {RSD^2}_D + {RSD^2}_{DS} + {RSD^2}_R} \quad (20)$$

T: is the content of (IB) and found equal to 98.92%,

%, then: $u_{(T)} = 98.92 \times$

$$\sqrt{\left(\frac{0.00136}{0.4214}\right)^2 + \left(\frac{0.00136}{0.4253}\right)^2 + \left(\frac{0.0163}{60.2}\right)^2 + \left(\frac{0.00289}{0.995}\right)^2 + 0.00383^2 + 0.011758^2 + 0.00436^2}$$

$$u_{(T)} = 1.40\%$$

On the other hand, using a coverage factor equal to 2, the expanded uncertainty is found equal to 2.8%. Hence the final result of the Irbesartan content is expressed as follows: $T = (98.9 \pm 2.8)\%$

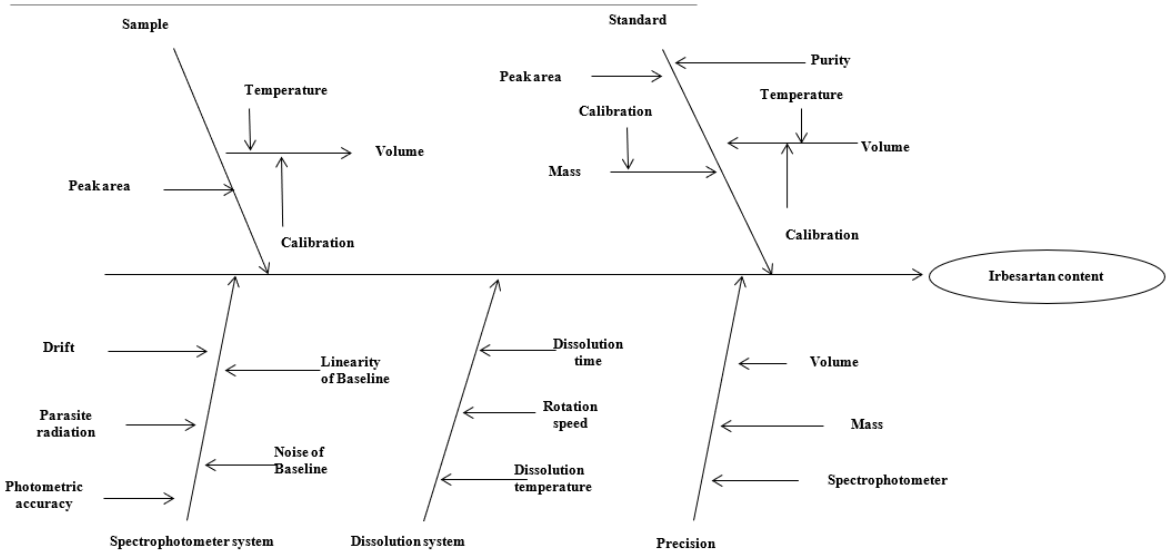

Fig. 5. Ishikawa diagram showing the causes and effects including the sources of uncertainty for the dissolution analysis procedure b2y UV/VIS Spectrometry

2.6. Evaluation of the uncertainty of measurement by the Simulation approach

The analytical approach GUM and numerical MCS have as common steps the identification of elementary sources of uncertainties and the design of the measurement model; however, they differ in their operating principles. Indeed, the principle of the MCS approach is to evaluate a compound uncertainty by the distribution propagation method.

The parameters that determine the PDF of each of the input quantities depend on the type of distribution. For a Gaussian PDF, the mean and its standard deviation are required. For rectangular and triangular distribution, the lower and upper limits a and b are needed. The PDF functions of different input quantities, for the two dissolution tests, are summarized in Tables 6 and 7. The information in these two tables makes it easy to calculate the mean, the standard deviation and the confidence interval of the output quantity by the propagation of these distributions by simulating a number of times the calculation of the output quantity which should have a Gaussian curve as shown in Figures 6 for Repaglinide content and Irbesartan content.

It is important to note that the number of trials M has a strong influence on the expected coverage probability of the output quantity. In this context, we selected a number M = 10^6 of simulations to generate the input values. This number is large enough to ensure the stability of the results in the statistical sense. The implementation of the MCS was carried out by the Matlab software via a program consisting mainly of the following functions:

$$NORMRND(mean, standard\ deviation, M, 1)$$

Which guarantees a normal distribution of the generated data.

And $\left(a + (b-a) \times RAND(M,1)\right)$ &

$$(a + \frac{(b-a)}{2} \times \left(RAND(M,1) + RAND(M,1)\right))$$

Which can generate random values respectively for a rectangular and triangular PDF.

Where a and b refer to the lower and upper limits of the distribution and the RAND function returns a random number greater than 0 and less than 1.

3. Discussion

Using the same input values and the same assumptions on the probability density functions of the input quantities, the simulation process is repeated a sufficiently large number of times to produce in the output a set of stable results.

The mean and standard deviation of the output results are then the respective estimates of the measurand (content) and its standard uncertainty. Indeed, we used 4 draws numbers M:

$$M = 10^5,\ M = 10^6,\ M = 1{,}5 \times 10^{6^6}$$
$$and\ \ M = 2 \times 10$$

then we opted for a reliable 95% coverage interval, as advocated by the Supplement 1to the GUM because the results keep their stability from $M = 10^6$ The

Figure 6 shows the histograms of the simulated results which are relatively symmetrical distributions, and several input quantities follow uniform probability laws, hence the final result resembles a normal distribution.

The results of the Monte-Carlo simulation for the two case studies are summarized in Table 8. Thus, the expanded uncertainty calculated using the coverage factor k = 2 (for 95% confidence assuming a Gaussian distribution) leading to a symmetric confidence interval, in contrast, the Monte-Carlo simulation gives an asymmetric confidence interval compatible with the actual distribution of the content and narrow than that of GUM. This narrowness is due to the values of the coverage factor k obtained by MCS which is relatively small compared to that assumed by the GUM method. They can be deducted for the two case studies as follows:

$k_{(RG)} = 2.3099/1.1854 = 1.9486$ and
$k_{(IB)} = 2.5589/1.3071 = 1.9577$

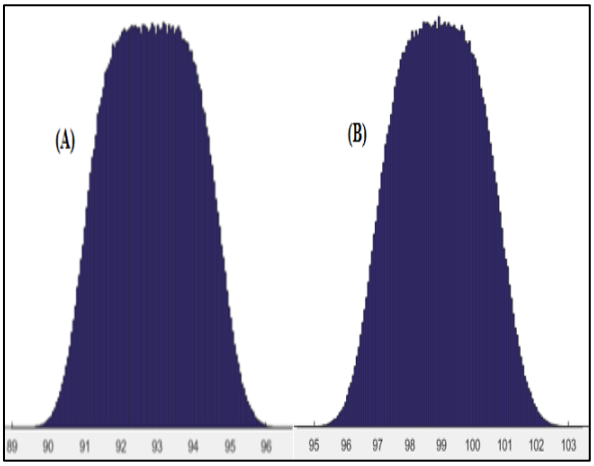

Fig.6. Distribution of the output quantity of the Repaglinide content (A) and Irbesartan content (B)

Table 6: Input parameters and their assigned PDF for the mathematical model of Repaglinide content in a tablet after a dissolution test.

Parameters	***Description***	***Type of Distribution***	***Mean***	***Standard deviation***	***a***	***b***
F_{rep}	Repeatability	Normal	1	0.00182	*	*
A_s	Area of the test solution	Normal	0.902	0.0016	*	*
A_{st}	Area of the standard solution	Normal	0.973	0.0016	*	*
W_{st}	weight of the reference substance	Rectangular	*	*	10.00	10.04
V_{C1}	Volume of 100mL	Triangular	*	*	99.9	100.1
V_{T1}		Rectangular	*	*	99.916	100.084
V_{C2}	Volume of 5 mL	Triangular	*	*	4.985	5.015
V_{T2}		Rectangular	*	*	4.9958	5.0042
V_{C3}	Volume of 100 mL	Triangular	*	*	99.9	100.1
V_{T3}		Rectangular	*	*	99.916	100.084
V_{C4}	Volume of 5 mL	Triangular	*	*	4.985	5.015
V_{T4}		Rectangular	*	*	4.9958	5.0042
V_{C5}	Volume of 50 mL	Triangular	*	*	49.94	50.06
V_{T5}		Rectangular	*	*	49.958	50.042
$V_{C'1}$	Volume of 1000 mL	Triangular	*	*	995	1005
$V_{T'1}$		Rectangular	*	*	999.16	1000.84
$V_{C'2}$	Volume of 10 mL	Triangular	*	*	9.975	10.025
$V_{T'2}$		Rectangular	*	*	9.9916	10.0084
$V_{C'3}$	Volume of 5 mL	Triangular	*	*	4.985	5.015
$V_{T'3}$		Rectangular	*	*	4.9958	5.0042
P	Purity of Repaglinide	Rectangular	*	*	0.9998	1
F_{DT}	Dissolution temperature	Rectangular	*	*	0.9945	1.0055
F_{Dt}	Dissolution time	Rectangular	*	*	0.9999	1.0000
F_{RS}	Rotation speed	Rectangular	*	*	0.98	1.02

Table 7: Input parameters and their assigned PDF for the mathematical model of Irbesartan content in a tablet after a dissolution test.

Parameters	*Description*	*Type of Distribution*	*Mean*	*Standard deviation*	*a*	*b*
F_{rep}	Repeatability	Normal	1	0.00186	*	*
DO_s	Optical density of0 the test solution	Normal	0.4214	0.00136	*	*
DO_{st}	the optical density of the standard solution	Normal	0.4253	0.00136	*	*
W_{st}	weight of the reference substance	Rectangular	*	*	60.18	60.22
V_{C1}	Volume of 100 mL	Triangular	*	*	99.9	100.1
V_{T1}		Rectangular	*	*	99. 916	100.084
V_{C2}	Volume of 2 mL	Triangular	*	*	1.99	2.01
V_{T2}		Rectangular	*	*	1.99832	2.00168
V_{C3}	Volume of 100 mL	Triangular	*	*	99.9	100.1
V_{T3}		Rectangular	*	*	99.916	100.084
$V_{C'1}$	Volume of 1000 mL	Triangular	*	*	995	1005
$V_{T'1}$		Rectangular	*	*	999. 16	100.084
$V_{C'2}$	Volume of 25 mL	Triangular	*	*	24.96	25.04
$V_{T'2}$		Rectangular	*	*	24.979	25.021
$V_{C'3}$	Volume of 2 mL	Triangular	*	*	1.99	2.01
$V_{T'3}$		Rectangular	*	*	1.99832	2.00168
P	Purity of Irbesartan	Rectangular	*	*	0.99	1
F_{DT}	Dissolution temperature	Triangular	*	*	0.9946	1.0054
F_{Dt}	Dissolution time	Triangular	*	*	0.999996	1.000004
F_{RS}	Rotation speed	Rectangular	*	*	0.98	1.02

Table 8 : Results obtained by the Monte-Carlo simulation.

Parameters	**Estimated values**	
	Repaglinide	**Irbesartan**
Mean (content%)	92.8631	98.9189
Median (%)	92.8595	98.9111
Standard uncertainty %	1.1854	1.3071
Expanded uncertainty	2.3099	2.5589
Skewness	0.0175	0.0168
Coverage factor k	1.9486	1.9577
Confidence interval for 95%	[90.464 ; 95.207]	[96.107 ; 101.697]

The results summarized in Table 9 clearly show that the values of the standard uncertainty obtained during the implementation of the numerical program correspond to values calculated algebraically without using the computer for both applications. In other words, there is not a significant difference between the parameters computed by the MCS approach, namely the estimated average value (T_{MCM} (IB) = 98.92 ; T_{MCM} (RG) = 92.86) , the combined uncertainty (u_{MCM} (IB) = 1.3071% ; u_{MCM} (RG) = 1.1854%), and those

calculated by the GUM approach (T_{GUM} (RG) = 92.86, T_{GUM} (IB) = 98.92 and u_{GUM} (IB) = 1.4028%; u_{GUM} (RG) = 1.1885%). This concordance of results from the two approaches is mainly due to the linear models in the two case studies.

Table 9: Comparison of the results obtained using the GUM approach and MCS for both case studies.

		Case study N°1		Case study N°2	
		GUM	**MCS**	**GUM**	**MCS**
Mean value		92.8632	92.8631	98.9162	98.9189
Standard uncertainty		1.1885	1.1854	1.4028	1.3071
Coverage factor		2	1.94863	2	1.9577
Expended uncertainty		2.3770	2.3099	2.8056	2.5589
coverage interval of 95%	Lower limit	90.486	90.464	96.111	96.107
	Upper limit	95.240	95.207	101.722	101.697

This observation taking us back to checking the concordance between these two approaches and confirming the validity of the results obtained by the application of the uncertainties propagation law (ISO-GUM approach). To do this, a computation of two differences d_{low} and d_{high} was carried out using the equation 11, and then their values were compared with the degree of approximation δ by checking the following condition: (d_{low} et d_{high}) < δ.

Thus, for the case of the Repaglinide dissolution test, L_{low}, L_{high}, d_{low} and d_{high} are respectively calculated as follows:

$$L_{low} = 92.863 - 2.377 = 90.486 \quad \&$$
$$L_{high} = 92.863 + 2.377 = 95.240$$
$$d_{low} = |90.486 - 90.464| = 0.02 \quad \&$$
$$d_{high} = 95.240 - 95.207 = 0.03$$

In addition, to obtain δ, the standard uncertainty u (T) = 1.2 with two significant digits can be written in the form, then $\delta = 1/2 \times 10^{-1} = 0.05$.

The entire calculation for both case studies is summarized in Table 10. Based on these results, the finding is that the criterion described by the supplement 1 is respected in the two study examples (since d_{low} and d_{high} <δ = 0.05).

As a consequence, we can conclude that the model developed and based on the law of propagation of the variances is valid. However, it is interesting to note that the choice of the numerical tolerance δ for the standard uncertainty and the coverage probability are subjective decisions and will influence the comparison of these two approaches [11, 22], because if u(T) is considered with a tolerance of three decimal places (δ = 0.005), the GUM and the MCS give statistically different results, and in this case the results obtained by the MCS method must be taken into account.

Table 10: Validation of results of the ISO-GUM approach by the Monte-Carlo simulation.

	GUM		MCS		D_{low}	D_{high}	δ
RG	y	92.863	Y_{low}	90.464	0.0220	0.0335	0.05
	U	2.377	Y_{high}	95.207			
IB	y	98.916	Y_{low}	96.107	0.0032	0.0245	0.05
	U	2.806	Y_{high}	101.697			

These results also show that when working in conditions where the measurement model is linear and the measurand (content or concentration) follows a normal law as in the case of the two examples studied, the laboratory can implement the ISO-GUM approach for estimating measurement uncertainty since it is a widespread and recognized method and does not require the use of a specific software, such as the Monte-Carlo method.

However, when the measurement models are complicated in particular the nonlinear models, from a practical point of view, the application of the GUM approach appears difficult, especially for calculating the derivatives in order to estimate the sensitivity coefficients. Moreover, this method supposes assumptions on the distributions of the input quantities and that the output quantity (measurand Y) must present a Gaussian distribution to justify the value taken from the coverage factor k [23].

Nevertheless, the Monte-Carlo simulation, on its part, can handle complicated model cases and provide extended intervals that do not require assumptions about the output quantity distribution. The MCS method allows not only spreads the mean and the variance but also the distributions of all the variables of the measurement process leading to reliable results of the estimates of uncertainty.

In the practice of pharmaceutical laboratories, several complex analysis instruments, such as HPLC, GC

LC-MS, GC-MS, SAA, are used in many analytical methods to analyze different pharmaceutical matrices

(liquid, solid, pasty form, etc.) Thus, the implementation of the two methods (GUM and MCS), in these cases, to estimate the measurement uncertainty is observed arduous and tedious. This finding, which generally came from the practice and particularly from this study, is justified either by an incorrect census of sources of uncertainty, by the difficulty of constructing the budget model of uncertainty or by the difficulty of evaluate uncertainty with the Type B method due to lack of information or implausible assumptions about the distributions of input quantities.

Adding that the lack of skills in metrology and calculation of uncertainty especially statistics, is sometimes considered as an obstacle to the implementation of the GUM method. For these reasons, scientific researchers in the pharmaceutical field, in particular the SFSTP group [9], have recently published a guide that offers solutions, based on the principles of the GUM method, but they use the type A method to estimate the measurement uncertainty of methods of drugs assay. Thus, the commission proposed to exploit analysis process control charts as recommended in ISO 11352 standard or to draw in a simple and rapid way the measurement uncertainty from the validation data carried out using the total error approach[24-26].

4. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

5. Acknowledgements

The authors are very grateful to the anonymous reviewers for providing important comments that led to significant improvements of this article.

6. Conclusion

This paper presents a comparative study of two universally recognized methods for estimating measurement uncertainty, namely the ISO-GUM approach (analytical method) and the Monte- Carlo simulation (numerical method).

After spreading the principles and steps of these two methods, we proceeded to apply them to the two dissolution tests: the first of Repaglinide with an assay carried out by HPLC and the second of Irbesartan whose assay is carried out with UV/VIS spectrophotometry. The extended intervals obtained by the two methods are then compared; it is what has enabled us to note that the uncertainty estimated with the two approaches showed no significant difference, and that the difference between the two confidence intervals obtained is very small. In other words, the MCS method is compatible with the analytical method for estimating uncertainty. This observation is perfectly plausible because the conditions of use of the GUM approach are fulfilled, in particular the simplicity of the measurement process model for the two examples treated and the normality of the distribution of the output measurand in this case the active ingredient content.

In addition, the results provided by the MCS method are considered more reliable because, in contrast to the GUM method, it requires practically no approximation of Taylor's development and gives a coverage interval from a PDF of the output quantity without making a Gaussian or other assumption about the shape of this distribution [6]. The result of this work shows that metrology knowledge could be better disseminated, the type of cases handled relating to a wide community of users of dissolutest apparatus, HPLC and UV/VIS Spectrometry allowing them to have a critical look.

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