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Evaluation of measurement uncertainty resulting from the determination of artesunate powder for injection content by a chromatographic technique (HPLC) at the National Quality Control Laboratory of the Democratic Republic of the Congo

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ABSTRACT

Introduction

To address the lack of data since the laboratory became operational in 1982, a collaboration was established with the National Quality Control Laboratory (NQCL) of the Democratic Republic of the Congo to evaluate the expanded measurement uncertainty associated with the assay of artesunate powder for injection prior to the routine application of the *International Pharmacopoeia*, 12th edition (2025) monograph.

Purpose

To evaluate the expanded uncertainty in accordance with normative requirements during the assay of artesunate powder for injection in order to verify the reliability of the quality control method intended for routine use.

Methods

The assay was performed according to *International Pharmacopoeia*, 12th edition (2025), Option A. Analysis was conducted by HPLC using a stainless-steel column (10 cm × 4.6 mm, 3 µm). The mobile phase consisted of 44 mL of acetonitrile R and 56 mL of phosphate buffer adjusted to pH 3.0. A quantity of the mixed contents containing approximately 40 mg of artesunate was accurately weighed. Peak response areas obtained from the chromatograms were measured, and the artesunate content was calculated (specification: 90.0–110.0%). The methodology was based on the *Guide to the Expression of Uncertainty in Measurement* (GUM), using a bottom-up approach grounded in intra-laboratory data. The measurement process was modelled through a 5M cause-and-effect analysis using an Ishikawa diagram, and uncertainty estimation was carried out using the law of propagation of uncertainty for Type A and Type B components. The parameters shown to have a significant impact on the uncertainty budget were: active pharmaceutical ingredient (API) weighing, HPLC IQ/OQ, volumetric measurements (volumetric flasks), and HPLC response (peak response areas).

Results

The artesunate content obtained during method verification was 100.3%, with an expanded uncertainty of 0.34%. Taking the calculated expanded uncertainty into account, the reported result was 100.3 ± 0.34%.

Conclusion

The expanded uncertainty was ±0.34%, and the overall result fell within the acceptable specification range. It can therefore be concluded that the *International Pharmacopoeia*, 12th edition (2025) method is reliable, as the result is close to the nominal value.

INTRODUCTION

Following the World Health Organization (WHO) recommendations issued in December 2015 after a review of the situation in the Democratic Republic of the Congo (DRC), no laboratory, including the national laboratory, was compliant with the normative requirements governing testing and calibration laboratories in accordance with ISO/IEC 17025:2017 (WHO, 2015). Any laboratory seeking to demonstrate recognised competence beyond its immediate sphere of influence must establish the reliability of its results by ensuring that the reported dosage value of the target analyte accounts for all potential errors (random and systematic), including measurement uncertainty.

When using the *International Pharmacopoeia* (*Ph. Int.*, 12th ed., 2025) for the quantitative analysis of artesunate powder for injection, Method A recommends chromatographic analysis. High-performance liquid chromatography (HPLC) is a widely used analytical technique in research and quality control laboratories, enabling the separation, identification, and quantification of sample components. To date, the National Quality Control Laboratory of the DRC has not fully implemented the requirements related to the evaluation of the reliability of its routinely applied analytical methods.

According to ISO/IEC 17025:2017, clause 7.6.1, "*the laboratory shall identify the contributions to measurement uncertainty and shall take into account all significant contributions, including those arising from sampling, using appropriate analytical methods*" (ISO/IEC, 2017). The estimation of expanded uncertainty (U) may be carried out using either a bottom-up or a top-down approach. Such estimation does not yield a single deterministic value but rather a range of values distributed around the measured result (Boudinet, 2020).

Widely recognised guidelines, including the *Guide to the Expression of Uncertainty in Measurement* (GUM) and the Eurachem/CITAC Guide *Quantifying Uncertainty in Analytical Measurement*, emphasise the importance of establishing and maintaining robust procedures for the evaluation of measurement uncertainty in analytical testing (JCGM, 2020).

Therefore, the primary objective of this study was to estimate the expanded uncertainty (U) associated with the result obtained during verification of the standard method for the assay of injectable artesunate, in accordance with regulatory requirements, by identifying and quantifying the influencing parameters.

METHODS

Reagents

Dihydrogen phosphate, 85% concentrated phosphoric acid, and HPLC-grade acetonitrile were supplied by Merck (Darmstadt, Germany). Artesunate reference standard (RS; batch 3/ICRS1409), artenimol RS (batch 2/ICRS1410), and artemisinin RS (batch 1/ICRS43857) were supplied by the *International Pharmacopoeia* through the European Directorate for the Quality of Medicines and HealthCare (EDQM; Strasbourg, France).

Ultrapure water was produced using an ECN171-1095 water purification system (VWR, France). Artesunate samples were obtained from the local market in Kinshasa and registered in the laboratory under reference number AQRe/CQP1524/10/2024. The samples were described as a fine, amorphous, white powder containing 120 mg per vial, sealed with rubber stoppers and secured with crimped aluminium caps. All samples analysed were provided by the Congolese Pharmaceutical Regulatory Authority.

Equipment

Qualitative and quantitative analyses were performed using an Agilent 1290 Infinity II HPLC-DAD system (Agilent Technologies, USA), operating as an ultra-high-performance liquid chromatography system (maximum pressure: 1300 bar) equipped with quaternary pumps. An L1 stainless-steel column (C18, 10 cm × 4.6 mm, 3 µm) was used.

Mettler Toledo analytical balances (ME204TE/00) and microbalances (XPR6UD5) were employed. An ultrasonic bath (Elmasonic; ServiLab, France) was used for solution homogenisation. Buffer pH was adjusted using a Metrohm 913 pH meter. Class A Pyrex volumetric glassware and 0.45 µm membrane filters were used throughout.

Sample Preparation

Sample preparation complied with Method A of the *International Pharmacopoeia* (12th ed., 2025).

Sample solution:
The contents of 10 containers were weighed. A quantity of the mixed contents containing approximately 40 mg of artesunate was accurately weighed and transferred into a 10 mL volumetric flask. Seven millilitres of solvent were added, the mixture was shaken to dissolve, diluted to volume, and filtered.

Standard solution:
Forty milligrams of artesunate RS were accurately weighed and dissolved in 10 mL of solvent.

System suitability solution:
Approximately 1 mg of artenimol RS, 1 mg of artemisinin RS, and 10 mg of artesunate RS were dissolved in 10 mL of solvent.

Chromatographic Conditions

The chromatographic conditions are summarised in **Table 1.**

Table 1:
Chromatographic conditions used for the assay of artesunate (International Pharmacopoeia, 12th ed., 2025).

Parameters	Artésunate
Column	C_{18},10cm ×4.6mm, 3µm
Mobile phase	44 volumes of acetonitrile R and 56 volumes of pH 3.0 buffer.
Flow rate	1.0 ml/min
Injection volume	20 µL
Column temperature	30°C
Wavelength	216nm
Resolution	N/A
Tailing factor (symmetry)	0.8 – 1.8
Relative standard deviation (for 5 injections)	0.73 – 1.10

Validation Parameters

Data processing was performed using Microsoft Excel 2016. Method performance characteristics were evaluated in accordance with WHO and ICH Q2(R1) guidelines prior to routine use (ICH, 2010; WHO, 2010).

Linearity:
Linearity was assessed over the range of 80–120% of the nominal concentration, with a coefficient of determination (R^2) ≥ 0.995.

Accuracy:
Accuracy was evaluated as the closeness of agreement between the measured value and the conventionally accepted true value across the same range. Bias did not exceed 2% for finished products.

Precision (repeatability):
Repeatability was evaluated using replicate analyses of homogeneous samples, with a relative standard deviation (RSD) not exceeding 2%.

Selectivity/Specificity:
The method demonstrated the ability to unequivocally identify artesunate in the presence of other components through comparison of sample and reference standard chromatographic peaks.

Calculation of the Measurement Uncertainty Budget

The uncertainty budget was established in accordance with GUM and Eurachem/CITAC guidelines (Eurachem/CITAC, 2025; JCGM, 2020). The following steps were applied: definition of the measurand, identification and grouping of uncertainty sources, quantification of components, conversion to standard uncertainties, calculation of combined uncertainty, and estimation of expanded uncertainty.

Analysis of Uncertainty Sources
An Ishikawa cause-and-effect diagram was used to identify and analyse the principal sources of uncertainty (Boilley, 2011; Farrance et al., 2018).

The assay result was calculated using the following equation:

$$\text{Result} = \frac{r_U}{r_S} \times \frac{C_S}{C_U} \times 100$$

where:

- rU is the peak response of the sample solution;
- rS is the peak response of the standard solution;
- CS is the concentration of artesunate RS in the standard solution (mg/mL);
- CU is the nominal concentration of artesunate in the sample solution (mg/mL).

The uncertainty of the final result was influenced by chromatographic performance (HPLC qualification), weighing of the active pharmaceutical ingredient, and volumetric measurements during solution preparation.

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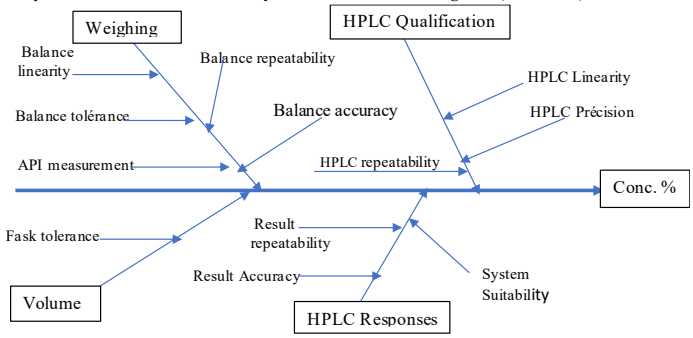
Figure 1:
Analysis of sources of uncertainty: Cause and effect diagram (Ishikawa)



Legend for Parameters and Expression of Standard Uncertainty

Uncertainties deduced from **Figure 1**:

Weighing (Balance):

- Balance tolerance: Type B uncertainty (uWt)
- Balance repeatability: Type A uncertainty (uWrep)
- Balance accuracy: Type B uncertainty (uWAc)
- Uncertainty of mass standard (uWms)
- Measurement (weighing) of artesunate (API): Weighing repeatability, Type A uncertainty (uWapi)

Volumetric flask (Volume):

- Flask accuracy: Type B uncertainty (uV)

HPLC Uncertainty Estimation:

- HPLC qualification (OQ) via repeatability: Type A uncertainty (uQ)
- Determination of API content: Repeatability of HPLC peak area responses, Type A uncertainty (uR)

These parameters were considered in the estimation of the expanded uncertainty related to the dosage of artesunate.

Note 1: Measurement uncertainty includes components arising from systematic effects, such as those associated with corrections and assigned values of standards, as well as definitional uncertainty.

Note 2: Some uncertainties were obtained through a Type A uncertainty assessment based on statistical distributions, while others were derived from a Type B uncertainty assessment.

Table 2:
Standard Uncertainty and Formulas Used (Protassov, 1999; ISO/IEC Guide 98-3, 2010)

Standard *Uncertainty*	Formulas Used
Repeatability Uncertainty (Type A)	$u_A = \frac{\sigma_{n-1}}{\sqrt{n}}$ (2)
Standard Uncertainty (B) Rectangular Distribution	u(t)=t/√ u(B)=$a/\sqrt{3}$ (3)
Standard Uncertainty (B) (u-tolerance) (4)	(4)
Standard Uncertainty (B) Triangular Distribution (X)	u(X) = a/√6 (5)
Combined Uncertainty X, Y,….Z	Uc=√(u(X)/X)²+ (u(Y)/Y)²....+ (u(Z)/Z)² (6)
Expanded Uncertainty (U) at 95% of coverage factor K= 2	U = Uc x K (7)

- Repeatability uncertainty (Type A)
- Standard uncertainty (Type B), rectangular distribution:
  $u(B) = a/\sqrt{3}$ (3)
- Standard uncertainty (Type B), tolerance-based (4)
- Standard uncertainty (Type B), triangular distribution:
  $u(X) = a/\sqrt{6}$ (5)
- Combined uncertainty (X, Y, …, Z):
  $U_c = \sqrt{(u(X)/X)^2 + (u(Y)/Y)^2 + \cdots + (u(Z)/Z)^2}$ (6)
- Expanded uncertainty (U) at 95% confidence level (coverage factor k = 2):
  $U = U_c \times k$ (7)

Table 2 provides the formulas corresponding to the two classes of uncertainty (Type A and Type B) used in the calculations.

Weighing Uncertainty, u(W)

Uncertainty in sample weighing (Ws = 40 mg) arises from the tolerance of the analytical balance, the accuracy of the weighing, and the uncertainty of the calibration standard.

1. The microbalance (XPR6UD5) used has a manufacturer-specified tolerance of ±0.0005 mg (0.5 µg). Using a rectangular distribution, the standard uncertainty related to the balance tolerance is:

u(Wt) = 0.0005 mg / √3 = 0.0002887 mg.

The corresponding relative uncertainty is:

ur(Wt) = u(Wt) / 5000 = 0.000000057735.

This calculation incorporates the uncertainty associated with the balance linearity during weighing.

2. The standard uncertainty related to repeatability was evaluated using a 5 g standard mass (Wms) subjected to 10 repeated measurements during balance calibration. The standard deviation obtained represents the repeatability uncertainty u(Wrep). The standard deviation from the calculations was 0.000001173787791 mg, and the calculated relative uncertainty is:

ur(Wrep) = 0.001173787791 mg / 5000 = 0.00000023475756.

3. Uncertainty related to balance accuracy was also considered. The nominal mass of the standard is 5.0 g, while the average measured mass during calibration was 5000.013833 mg. The standard uncertainty is calculated as:

u(WAc) = (5000.0 mg − 5000.013833 mg) / (2√3) = 0.0119797294 mg.

The corresponding relative uncertainty is:

ur(WAc) = u(WAc) / 5 = 0.0023959458821.

4. The uncertainty related to the mass standard was included due to its influence on the reliability of weighing values. According to archived metrological data, the expanded uncertainty at 95% confidence with a coverage factor k = 2 is 0.0005 mg. Since $U(x) = k \times u(x)$, the standard uncertainty is:

u(Wms) = 0.0005 / 2 = 0.00025 mg.

The corresponding relative uncertainty is:

ur(Wms) = 0.00025 mg / 5000 = 0.00000005.

Uncertainty Related to the Volume of the Volumetric Flask, u(V)
The standard uncertainty related to volume was estimated based on the manufacturer's specification of a 10 ± 0.02 mL volumetric flask. A triangular distribution was assumed:

u(V) = 0.02 mL / √6 = 0.0081649658 mL (GU-ISO 8655, 2022).

The relative standard uncertainty is:

ur(V) = 0.0081649658 mL / 10 = 0.0008164965809.

Uncertainty Related to Qualification of Analytical Equipment (HPLC), u(Q)
The standard uncertainty related to HPLC accuracy was estimated using a caffeine solution prepared at a concentration of 0.5 mg/mL. According to the most recent operational qualification (OQ) report, repeatability was assessed based on six injections of 20 µL each, which is directly relevant to the dosage procedure using caffeine as a reference standard (Hubert et al., 2016; EDQM, 2023).

The accuracy obtained during OQ was 0.04%. Assuming a triangular distribution, the standard uncertainty of repeatability is:

u(Q) = 0.04 / √6 = 0.0163299316.

The relative uncertainty is:

ur(Q) = 0.0163299316 / 20 = 0.0008164965809.

This estimation assumes that uncertainties related to other parameters not considered separately (such as flow rate, temperature, and response linearity) are implicitly included.

Uncertainty Related to Weighing of the Active Pharmaceutical Ingredient (API)
At this level, two major sources of uncertainty were considered. The uncertainty related to balance tolerance had already been accounted for. The remaining source concerns the repeatability of weighing the active ingredient (artesunate) used to prepare the test solution in accordance with the monograph (see **the section on Sample Preparation**), specifically the weighing of 40 mg of artesunate.

The standard deviation obtained represents the standard uncertainty:

u(Wapi) = 0.0186814 mg.

The corresponding relative uncertainty is:

ur(Wapi) = 0.0186814 mg / 40 = 0.0004670348627.

Uncertainty Related to Measurement of the Active Ingredient by HPLC, u(R)
This uncertainty was estimated from the standard deviation of the mean peak area responses obtained from three injections for each of three tests. The standard uncertainty obtained is:

u(R) = 0.04673053962%.

The corresponding relative uncertainty is:

ur(R) = 0.04673053962 / 40 = 0.0011668263491.

Standard Uncertainty of the Artesunate Content

The combined relative standard uncertainty was calculated by summing the squares of the individual relative uncertainties of each contributing component, in accordance with GUM principles (ISO/IEC Guide 98-3, 2010; Protassov, 1999). The expanded uncertainty was calculated using a coverage factor $k = 2$, corresponding to a confidence level of approximately 95% (JCGM, 2020). The calculated expanded uncertainty was 0.34%. Therefore, the value attributable to the artesunate content of the sample was: **100.3 ± 0.34%**

RESULTS

Artesunate Assay

Analytical results were obtained from three replicate injections under the chromatographic conditions described in **Table 1**.

Table 3:
Artesunate Assay Results

	Average Rs /Areas	Rs Concentrations mg/mL	Sample/ Areas	Sample concentration Averages	Content (%)	SD	RSD (%)	Relative Bias (%)	Linearity R^2
			2782.996	4.0081	100.2023			-0.2023	
	2777.3765	4.0000	2784.422	4.0101	100.2537	0.00185	0.04667	-0.2537	0,997
			2785.586	4.0118	100.2956			-0.2956	
Moyenne			2784.335	4,0100	100.2505			-0.2505	

As specific system suitability criteria are not explicitly stated for artesunate in the monograph, the general system suitability requirements of the *International Pharmacopoeia* were verified prior to assay. These included comparison of the retention time of the artesunate peak in the sample (9 min) with that of the reference standard (9 min) and with related substances (artenimol and artemisinin), as described in the analytical procedure. Method pre-verification in accordance with ICH Q1(R2) and WHO requirements was successfully completed, as summarised in **Table 3** (ICH, 2005; WHO, 2010).

Table 3 presents the results of the evaluation of validation parameters, including linearity (R^2), accuracy (relative bias), reliability (repeatability expressed as RSD), and selectivity/specificity (unequivocal peak identification).

The principal parameters considered in the uncertainty budget are summarised in **Table 4**.

Table 4:
Results of Overall Uncertainty Estimates Related to Artesunate Assay by HPLC

A) BALANCE		Calculations	Parameter Values	Standard Uncertainties	Assigned Values	Units	Relative standard Uncertainty
1)Balance precision		d=0.00005mg	0.00005	0.0000002887	5000	mg	0.000000057735
2) Balance Repeatability (5g x 10 repetitions)		5000,016 5000,013 5000,014 5000,013 5000,013 5000,015 5000,014 5000,013 5000,013 5000,012			5000	mg	0,0000002348
	Mean	5000,0136					
	SD	0,0011737877908	0,001173787791	0,0011737878			
3) Accuracy (accuracy-balance error) at 5g (result = 5.01383 g	5000 - 5000,013833(mg)	0.0138330000	0.0138330000	0.0119797294	5000	mg	0.0000023959459
4) Standard uncertainty (5g)	Extended uncertainty (U), K=2 at 95%)	0.0005000000	0.000500000000	0.0002500000	5000	mg	0.0000000500000

B) VOLUME FLASK (Flask Accuracy 10±0.02 mL)		Accuracy=0.02mL	0.02	0.0081649658	10	mL	0.0008164965809
C) HPLC UNCERTAINTY ESTIMATE Qualification (Area Repeatability for 6 injections x 20 µL)		Accuracy=0.04µL	0.04	0.0163299316	20	µL	0.0008164965809
D) MEASUREMENT (Weighing) of Artesunate	Test sample (Artesunate, n=3)	Value obtained (monograph's value = 40mg/10mL)					
	Mesure 1	40.08093681			40	mg	0.0004670348627
	Mesure 2	40.10146266		0.0186813945			
	Mesure 3	40.11823676					
	Mean	40.10021208					
	SD	0.01868139451					
Incertitude de Répétabilité	Uncertainty Type	0.01868139451					
E) DETERMINATION OF API CONTENT (%)	Mesure 1	100.2023			40	mg	0.0011668263491
	Mesure 2	100.2537		0.04667305396			
	Mesure 3	100.2956					
	Mean	100.2505333					
	SD	0.04667305396					
	Uncertainty Type	0.04667305396					

Table 4 summarises the parameters with a probable contribution to the uncertainty associated with the assay of artesunate powder for injection.

Estimation of the Expanded Uncertainty Related to Artesunate Assay

The combined and expanded uncertainties associated with the artesunate assay were calculated in accordance with GUM and Eurachem/CITAC guidelines (JCGM, 2020; Eurachem/CITAC, 2025). A summary of the uncertainty contributions is presented in **Table 5**.

Table 5:
Summary of Overall Uncertainty Estimates for Artesunate Determination by HPLC

Description	**Standard Uncertainty *u(x)***	**Square of Relative Standard Uncertainty *(u(x)/x)²***	**Contribution (%)**
1) Balance precision	0,00000005773503	0,0000000000000	0,00
2) Balance repeatability (5 g standard)	0,0000002347576	0,0000000000001	0,00
3) Balance accuracy (standard = 5 g)	0,00000239594588	0,0000000000057	0,00
4) Uncertainty of the 5 g standard	0,00000005000000	0,0000000000000	0,00
5) Flask accuracy	0,00081649658093	0,0000006666667	22,89
6) Equipment qualification (HPLC)	0,00081649658093	0,0000006666667	22,89
7) Measurement (test portion)	0,00046703486267	0,0000002181216	7,49
8) Determination of artesunate content	0,00116682634905	0,0000013614837	46,74
Sum of squares		**0,0000029129445**	
Square root of sum of squares, U_c		**0,001706735**	
Uncertainty of artesunate content (100,2505%)		**0,17110104**	
Coverage factor (k = 2, 95%)		**2**	
Expanded uncertainty (U)		**0,34220208**	
Rounded expanded uncertainty		**0,34**	

Table 5 provides a summary of the calculations used to estimate the expanded uncertainty at a confidence level of 95%, with a coverage factor k = 2.

The relative contribution of each parameter to the overall uncertainty budget is illustrated in Figure 2.

Figure 2:
Contribution of Each Parameter to the Uncertainty Budget

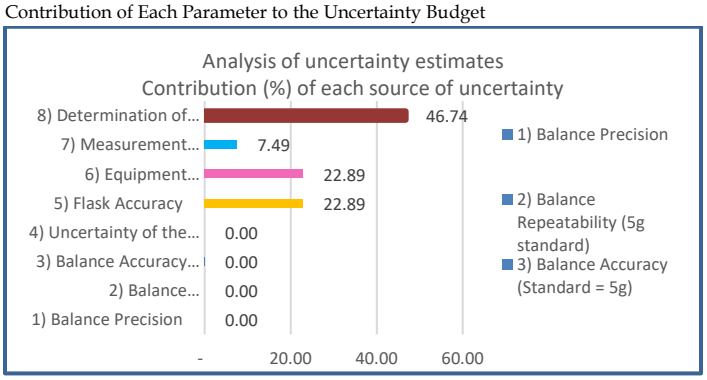


Figure 2 illustrates the percentage contribution of each identified source of uncertainty included in the expanded uncertainty estimation.

DISCUSSION

The results demonstrate that the chromatographic system complied with the specifications of the pharmacopoeial method used. The standard deviation (SD), relative standard deviation (RSD), relative bias, and linearity values were consistent with acceptance criteria. The expanded uncertainty obtained (U, k = 2) was ±0.34%, and the value attributable to the artesunate assay was 100.3 ± 0.34%.

This study was initiated to assess the uncertainty associated with measurements performed during the assay process (Farrance et al., 2018; Kim et al., 2023) and to ensure the reliability and accuracy of the final result obtained for the active pharmaceutical ingredient (API) content (Andanson et al., 2019; Kim et al., 2023; Oosterhuis et al., 2018). The estimation of expanded uncertainty was based on cause–effect analysis using an Ishikawa diagram **(Figure 1)** and the bottom-up approach, incorporating validation data, as recommended in the literature (Taylor et al., 2022). These results are summarised in Tables 4 and 5 and illustrated in Figures 1 and 2.

The most significant contributions to the uncertainty budget, in descending order, were attributed to determination of API content (46.74%), HPLC equipment qualification (22.89%), volumetric flask accuracy (22.89%), and API weighing (7.49%). These substantial contributions may be related to limited knowledge of systematic bias, which can introduce additional uncertainty into the measurement process.

These findings confirm that an assay result cannot be considered complete without the associated expanded uncertainty, which reflects the cumulative contribution of multiple influencing factors, as shown in Figure 2. A low uncertainty value indicates that both systematic and random errors are minimal and that the result is close to the true value. Consequently, medicines tested using this analytical method can be considered reliable for regulatory authorities and end users, reinforcing public confidence in the laboratory's capacity to detect counterfeit medicines using validated and reliable methods.

Although no similar studies on artesunate powder for injection were identified in the literature, comparable studies have been reported for other analytes. For example, Bozdayi et al. (2025) compared measurement uncertainties associated with two analytical methods for ethanol determination, reporting uncertainty values within ±20% in accordance with ISO/TS 20914. Similarly, Sumita et al. (2024) evaluated uncertainty in the determination of boron using PGA and ICP-OES, obtaining consistent results when uncertainty was considered. In medicinal chemistry, Lee and Kim (2022) applied a similar validation and uncertainty evaluation approach to the analysis of piperine in black pepper. Furthermore, Kim et al. (2023) assessed uncertainty in the quantification of urinary amphetamines, reinforcing the importance of uncertainty evaluation in analytical chemistry.

It should be emphasised that the selection of materials and preparation of solutions must be performed rigorously, and that analytical equipment must undergo appropriate qualification and calibration in order to minimise deviations (ISO/IEC, 2017; WHO, 2010). These considerations highlight the importance of systematic evaluation and careful control of potential sources of error in measurement processes.

Although not all possible sources of uncertainty were included in this study, emphasis was placed on those with

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the most significant and direct impact on the analytical result.

CONCLUSION

Based on this study, the necessity of implementing a robust quality management system, applying control charts for analytical data, and ensuring traceability of results has been clearly demonstrated. These practices are essential for compliance with regulatory requirements and for minimising random and systematic errors.

The bottom-up approach used to estimate the expanded uncertainty associated with the HPLC assay of artesunate, in accordance with the *International Pharmacopoeia*, proved to be practical and effective for laboratories seeking international recognition. Adherence to good laboratory practices—including proper qualification and calibration of equipment and rigorous preparation of solutions—resulted in a low expanded uncertainty (±0.34%), ensuring that the final assay result (100.3 ± 0.34%) remained within the compliance limits of 90.0–110.0%.

Limitations

Given the large number of potential sources of uncertainty, only those parameters with a direct and significant impact on the assay result were considered in this study.

Authors' Contributions: Michel Ntambwe Ngoyi: Conceptualisation, Investigation, Formal analysis, Writing – original draft. Béni Bisuta Lifayifi: Investigation, Formal analysis, Data curation. Jérémie Mbinze Kindenge: Investigation, Resources, Supervision, Visualisation. Roland Marini Djang'ieng'a: Methodology, Validation, Supervision, Strategy, Visualisation.

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