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APPLICATION OF THE VARIABILITY BUDGET APPROACH TO THE DISSOLUTION TEST

Dmytro Leontiev, Vitalii Asmolov, Natalia Volovyk, Oleksandr Gryzodub

Aim. *This study aimed to evaluate the completeness of our knowledge about the sources of variation in the Dissolution test with 100 % release by compiling a variability budget.*

Materials and methods. *The study was performed on 500 mg metformin tablets, using pharmacopoeial quality reagents, State Pharmacopoeia of Ukraine (SPhU) Metformin HCl reference standard, Pharmatest DT70 Dissolution apparatus, Perkin Elmer Lambda 35 spectrophotometer, Mettler Toledo XP 204 analytical balance, and ISO class A volumetric glassware. The SPhU metrological approach was employed.*

Results and discussion. *The variability budget was compiled based on the comparison of uncertainty estimates obtained from the requirements for maximum permissible variation in normal analytical practice (U^{NAP}, bottom-up estimation) and experimental data (U^{exp}). This involved characterizing Metformin content in tablets using the Uniformity of Dosage Units (UDU) test as an independent method. The 100 % release of Metformin in the Dissolution test (infinity point) was proved by increasing the dissolution time. Having optimized Dissolution and UDU analytical procedures for variability budget compiling, we achieved insignificance of U^{exp} compared to the target uncertainty (U^{tg}) for the Dissolution test in compliance testing. The differences in UDU and Dissolution mean results did not exceed U^{NAP} for the release time of 45 and 60 min, i.e. uncertainty budget was proven. U^{exp} for the Dissolution test indicated the presence of an unknown statistically significant source of random variation, which, however, was less than U^{tg}; therefore, the procedure is suitable for compliance testing.*

Conclusion. *Experimental results confirmed the completeness of our knowledge about sources of variation (absence of bias) for the Dissolution test with 100 % release. An essential condition for compiling the budget was the optimization of uncertainty of analytical procedures. For UDU, all significant sources of variation were within the expected range. Yet, there is a need for additional research to identify and manage an unknown source of practically significant random variation for the Dissolution test*

Keywords: *AQbD, target uncertainty, variability source, insignificance, normal analytical practice, dissolution, infinity point, uniformity of dosage form, metformin tablets, variability budget*

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1. Introduction

1. 1. Quality assurance of medicinal products and the concept of measurement uncertainty

Ensuring the quality of medicines is a paramount concern in pharmacy. For successful quality assurance of any pharmaceutical product, the reliability of analysis results is essential. These results must provide sufficient confidence to make reliable decisions regarding compliance with specifications [1]. The Analytical Quality by Design (AQbD) approach emphasizes ensuring the quality of analytical results by comprehensively understanding the sources of variation and subsequently controlling them [2, 3]. Meanwhile, the concept of measurement uncertainty is a contemporary scientific paradigm that associates the influence of variation sources on analysis results with the risk of making incorrect decisions [4]. The uncertainty associated with a result is a fundamental indicator of its reliability for decision-making, as documented in several publications by the United States Pharmacopoeia (USP) [5–7].

To evaluate the risk of making an incorrect decision on compliance, one must consider acceptance criteria, focusing primarily on the established target uncertainty (U^{tg}) for relevant pharmaceutical product tests. Currently, the world's leading pharmacopoeias mainly delve into the rationale for U^{tg}, as seen in the MHRA [8] and the USP [4, 9]. However, specific recommendations concerning U^{tg} for standard pharmacopoeial tests are uniquely outlined in the State Pharmacopoeia of Ukraine (SPhU) [10, 11]; hence, when evaluating the risk of making an incorrect compliance decision, it is prudent to refer to them.

An integral aspect of analysis result variability is bias, which pertains to the performance characteristic of accuracy [9]. Contrary to the precision component of variability, bias cannot be minimized merely by averaging analysis results. Consequently, it is crucial to understand and control bias to maintain the reliability of the analysis results [7].

1. 2. Variability budget composition: solid dosage units

Solid dosage units (SDU) are the most prevalent finished products. However, analyzing the variability sources of SDU is particularly challenging due to the

complex technology involved in SDU preparation, which hinders the production of a model SDU with known analyte content [8], and the heterogeneity in analyte content between individual SDU units [12].

Building on AQbD principles and the concept of uncertainty in line with the SPhU approaches [10, 11], we introduced the variability budget approach [13]. This approach entails:

– establishing an assigned content value for the analyte in the object being analyzed based on independent information;

– estimating the amplitude of variation (or uncertainty) for all identified significant variability sources for the specific test under consideration;

– conducting a bottom-up evaluation of total variation;

– running the designated test and checking whether (1) the variation in analysis results exceeds the uncertainty estimate (precision) and (2) the mean value's deviation from the assigned value lies within the estimated uncertainty (bias).

This approach aims to verify the completeness of our understanding of knowledge about major variability sources. Without such verification, one cannot vouch for reliable process management. Importantly, this variability budgeting strategy offers the potential to regulate both the precision and accuracy (bias) of analysis results.

We previously applied this variability budgeting strategy when devising the transfer procedure for the assay of desloratadine tablets. The knowledge gained about significant variability amplitudes [14, 15] informed the criteria for transfer result acceptability, factoring in technological and analytical variability sources and variations influencing the precision and bias in the uncertainty components of the analysis result [13]. To our knowledge, no other studies have adopted a variability budget approach, possibly because requirements for U^{fg} guidelines are exclusive to the SPhU.

1. 3. Challenges in a variability budget composition for the Dissolution test

The Dissolution test [16] is pivotal in gauging the efficacy of SDU. Compared to the Assay, the Dissolution test incorporates additional specific variability sources, primarily due to the emergence of a new parameter – the extent of release of the active pharmaceutical ingredient (API) from an SDU unit and the introduction of additional equipment – the Dissolution apparatus, which, though not directly tied to measurements, significantly influences analysis results [17, 18].

Some scholarly papers scrutinize the variability sources and their amplitude for the Dissolution test [19] or evaluate its combined uncertainty [20]. Yet, no studies validate the comprehensiveness of knowledge about variability sources, rendering the variability budget approach for the Dissolution test particularly pertinent. One might argue that devising such a budget is the logical progression after understanding the principal variability sources and quantifying their variation.

To ensure the quality of Dissolution test results, the SPhU has stipulated U^{fg} for this test and proposed a measurement operation validation procedure [21]. Notably, in the SPhU, all validation acceptance criteria are explicitly defined and directly linked to the U^{fg} for this test [10, 11], i.e. the SPhU's metrological conception heavily leans on the uncertainty concept. However, the approach described in the SPhU doesn't consider the variability sources associated with the Dissolution apparatus.

Interestingly, the USP endorses an approach reminiscent of composing a variability budget for the Dissolution test [18]. This involves comparing 100 % release results (infinity point) with those from the Dosage Unit Uniformity (UDU) test, providing an independent assessment of the dosage form's analyte content. Nevertheless, this approach's practical implementation demands criteria and experimental design development, both lacking in USP recommendations.

The USP has formulated a test employing the USP Prednisone Tablet Reference Standard for PVT to regulate the pivotal variability source – the analyte's release rate – with a standardized release rate near 70 % [22]. Yet, when the release rate is substantially below 100 %, many additional variability sources can profoundly skew the results [23]. No prior studies have holistically examined the knowledge completeness regarding variability sources tied to the dissolution apparatus.

Consequently, we posit that achieving reliable control over a release rate considerably below 100 % remains elusive unless control over all variability sources unrelated to incomplete release has been previously established.

The study aims to verify the knowledge completeness of variability sources for the Dissolution test (for a 100 % release analysis object) by formulating a variability budget and applying the SPhU's metrological concept for experimental criteria and design. The subsequent tasks are imperative to attain this aim:

– propose metrological acceptance criteria and experiment design;

– identify the most suitable analysis object, which minimizes variability source impacts specific to the object on analysis results;

– optimize the analytical procedures (Dissolution and UDU) tailored to the task of variability budget composition;

– validate the compiled variability budget for the selected analysis object.

2. Planning of the research

The experiment design should aim at clarifying the nature of sources of variation. Criteria for result acceptance should be rooted in risk analysis; specifically, the confidence in a correct compliance decision should not be lower than 95 %. The criteria and result evaluation should employ metrological approaches of the SPhU [10, 11].

2. 1. Analysis methods and pharmacopoeial tests selection

The research employs the Dissolution Pharmacopoeial Test (Ph. Eur. 2.9.3) and, as an independent me-

thod of analysis, the UDU test (Ph. Eur. 2.9.40). Considering the extensive scope of the experiment, spectrophotometry is used for these tests (Ph. Eur. 2.2.25). Both tests should be performed under the same analysis conditions to facilitate the establishment of the variability budget. Analytical procedures are optimized to attain the required uncertainty level.

2. 2. Selection of the object of analysis, experimental design, and criteria

The choice of analysis object, experimental design, and criteria are intrinsically intertwined, warranting a joint consideration.

The experimental design, in conjunction with the choice of the analysis object, should minimize the influence of known sources of variation to an acceptable level and be able to detect unexpected ones effectively. The known variation sources influenced by the experiment's design and the analysis object selection include heterogeneity of analyte release between different SDU units, resulting in random variation in the analyte concentration of test solutions and result variations stemming from standard operations in the analytical procedure, including sample preparation and measurement.

Several factors contribute to the noted heterogeneity in the analyte release across SDU units:

– variation in the individual tablet weight;

– heterogeneity of the tablet mass in terms of the analyte content;

– differences in the release rate from individual tablets.

Release calculations for the Dissolution test are conducted in mg/mg of the tablet mass, requiring the weighing of each tablet before analysis to mitigate the impact of variations in individual tablet mass. The effect of a tablet shell not containing an analyte introduces bias in terms of mg/mg tablet weight calculations. An object of analysis, such as a film-coated tablet, is deemed suitable if it is demonstrated that the tablet coating's weight does not significantly impact analysis results. Following our previously established approach, an analysis object is chosen in which the inhomogeneity of tablet mass is theoretically low, based on a high API-to-excipients mass ratio [24]. The UDU test independently confirms this assumption, calculating the analyte content per tablet in mg/mg of individual tablet weight.

We follow the USP recommendations to avoid result variations due to incomplete analyte release into the dissolution medium [18]. This involves verifying 100 % analyte release under the selected dissolution test conditions, possibly by increasing the release time or stirring intensity.

Lastly, to account for the result variations caused by the analytical procedure's standard operations, optimization follows the SPhU's bottom-up uncertainty estimation approach for analysis results in the case of minimal compliance with pharmacopoeial requirements (the concept of normal analytical practice, NAP) [11, 13, 25].

2. 3. Proposed experiment design and analysis object selection

If necessary, study the stability of test and reference solutions over a sufficiently long period of time, applying suitable acceptance criteria for variability budget analysis.

Optimize the Dissolution and UDU analytical procedures, encompassing weighing, dilution, and measurement, so that the bottom-up estimated uncertainty, according to NAP, remains within the predefined threshold of U^{lg} for the variability budget.

Determine the analyte content in at least 30 tablets using the optimized UDU test method, which corresponds to UDU Level *L*2. From these results, we should:

– confirm the uniformity of tablet mass based on individual mg/mg tablet mass measurements;

– establish the average API content in the batch, calculated in mg/mg tablet weight.

Analyze at least 24 tablets of the selected object using the optimized Dissolution procedure (corresponds to Dissolution Level S_3) and assess the analyte's release in mg/mg of individual tablet mass.

Increase the dissolution time or stirring intensity to ensure complete analyte release (reaching the "infinity point" condition).

Estimate the variability budget: The average amount of API released in the Dissolution test should align with the batch's average API content determined by the UDU test (mg API/mg tablet mass) within the accepted criteria.

3. Materials and methods

Object of analysis: we selected Metformin film-coated tablets (500 mg) manufactured by Pharmex Group LLC, Ukraine, for our analysis. Each tablet contains 500 mg of Metformin HCl, 41.5 mg of core excipients, and a 9.8 mg shell.

Instrumentation and measurement:

– spectrophotometry: measurements were conducted using a Perkin Elmer Lambda 35 spectrophotometer. The wavelength was set at 233 nm, employing 1 cm quartz cuvettes. Each solution underwent three measurements (SPhU, $2.2.26^{N}$). The spectrophotometer was qualified as per Ph. Eur. 2.2.25 [26] and the OMCL quality management documents [27], ensuring adherence to international standards;

– dissolution test: a Pharmatest DT70 dissolution apparatus was employed. Mechanical qualification of the apparatus was performed following the recommendations of the FDA [28] and ASTM [29];

– analytical balance: a Mettler Toledo XP 204 analytical balance was used for precise measurements;

– reference standard: the State Pharmacopoeia of Ukraine Metformin HCl reference standard (SPhU Metformin HCl RS) was used, with the target uncertainty of the assigned value (U^{lg}) of 0.5 %, expressed as a 95 % one-sided confidence interval;

– volumetric glassware: all volumetric measurements were conducted using ISO class A glassware,

which was verified in our laboratory to ensure accuracy in volume measurements;

– reagents: all chemical reagents employed in this study were of pharmacopoeial qualification;

– solution filtration was performed using a PES (Polyethersulfone) or PTFE (Polytetrafluoroethylene) filter with a pore size of 0.45 μm;

– statistical analysis: the statistical analysis of our results was conducted, applying the SPhU's concept of uncertainty [30]. We used Excel software for all statistical calculations;

– solvent and dissolution media: pH 6.8 Phosphate buffer (the Ph. Eur. 5.17.1).

Analytical procedure for UDU test: the assay procedure was adopted and validated for the UDU test (Ph. Eur. 2.9.40 [31]).

Analytical procedure for routine quality control:

1. Test solution. Place 1 tablet into a 500.0 mL volumetric flask. Add 70 mL of solvent to the flask. Shake the flask for 15 min. Dilute the solution to a volume with the solvent. Filter the solution to remove any undissolved particles. Then, take 1.0 mL of this filtered solution and dilute it to 100.0 mL in a separate volumetric flask.

2. Reference solution. Dissolve 100.0 mg of SPhU Metformin HCl RS in 10 mL of the solvent. Dilute this solution to 100.0 mL with the solvent. Take 1.0 mL of the resulting solution and dilute it to 100.0 mL.

3. Analytical procedure for the variability budget check. All aliquots were weighed for an accurate calculation of the final concentration.

4. Test solution. Individually weigh each Metformin tablet. Place the weighed tablet in a 500.0 mL volumetric flask. Add 70 mL of the solvent to the flask. Shake the flask for 15 minutes. Dilute the mixture to a volume of 500.0 mL using the solvent. Filter the solution. Take 2.0 mL of this filtered solution and dilute it to 200.0 mL.

5. Reference solution. Dissolve 200.0 mg of SPhU Metformin HCl RS in 20 mL of the solvent. Dilute this solution to a total volume of 200.0 mL with the solvent. Take 2.0 mL of this solution and dilute it to 200.0 mL.

Analytical procedure for dissolution test: A total volume of 900 mL of dissolution media is used for the dissolution test.

The dissolution test is carried out using Apparatus 1 (basket apparatus) as specified in Ph. Eur. 2.9.3. The basket rotation speed is set at 100 rpm. The duration for the dissolution test is fixed at 45 min and, additionally, at 60 min (for the 100 % release confirmation — the "infinity point" approach [18]). When the time is reached, samples are withdrawn from the dissolution vessel. These samples are immediately filtered to ensure clarity and remove undissolved particles.

Analytical procedure for routine quality control:

1. Test solution. Take 2.0 mL of the solution from the dissolution vessel, filter it, and dilute it to a total volume of 200.0 mL.

2. Reference solution. Dissolve 55.6 mg of SPhU Metformin HCl RS in 10 mL of the solvent and dilute to a volume of 100.0 mL in a volumetric flask. Take a 2.0 mL sample of the solution and dilute it to 200.0 mL.

3. Analytical procedure for the variability budget check. All aliquots were weighed for an accurate calculation of the final concentration.

4. Test solution. Accurately weigh an individual Metformin tablet. Take 2.0 mL of the solution obtained from the dissolution vessel, filter it, and dilute it to a final volume of 200.0 mL.

5. Reference solution. Dissolve about 100.0 mg of SPhU Metformin HCl RS in 10 mL of the solvent and dilute the solution to 200.0 mL in a volumetric flask. Take a 2.0 mL sample of the solution and dilute it to 200.0 mL.

4. Results and discussion

4. 1. Criteria for establishing the variability budget

Following the SPhU approach, U^{tg} for the results of an individual unit analysis of the finished pharmaceutical product in both Dissolution and UDU tests is defined as [11, 21]:

$$U^{tg}=3.0\ \%. \tag{1}$$

It is proposed that for establishing the variability budget, the maximum acceptable uncertainty $\left(U^{tg}_{budget}\right)$, estimated in accordance with NAP requirements, should be insignificant compared to U^{tg} for both Dissolution and UDU tests in compliance testing. The value U^{tg}_{budget} is justified following the SPhU's principle of insignificance, leading to:

$$U^{tg}_{budget}=0.32\times 3.0\ \%=0.96\ \%. \tag{2}$$

A two-level criterion is suggested to check the variability budget effectively:

1. Estimated uncertainty of average analysis results.

Calculate the combined estimated uncertainty $\left(U^{fact}_{NAP}\right)$ of the average analysis results from the Dissolution and UDU tests, following the bottom-up approach [32] as per NAP requirements [10, 11]. The difference between the average results for the Dissolution test (X_{Dissol}) and the UDU test (X_{UDU}) should not exceed U^{fact}_{NAP}:

$$\left|X_{UDU}-X_{Dissol}\right|\le U^{fact}_{NAP}. \tag{3}$$

However, if U^{fact}_{NAP} is significantly less than the critical value of 0.96 % (equation 2), it could lead to non-fulfilment of the requirements for drawing up the variability budget as per equation (3). Hence, the second criterion described below is applied.

2. Acceptance regardless of U^{fact}_{NAP} value.

If the difference as per equation (3) is less than 0.5 %, the variability budget is considered met regardless of U^{fact}_{NAP} value. This criterion acknowledges the practical limitations in controlling variability sources below a 0.5 % threshold at the NAP level, which laboratories must meet [30].

Additionally, the criterion for the insignificant influence of the tablet shell on API determination results, in terms of an individual tablet's weight, can be formulated as follows. For a homogeneous tablet mass, a linear relationship should be observed between the analyte content in the tablet (X_{API}) and the tablet's weight (m),

where the shell weight contributes systematically, characterized by the intercept (a) in the linear equation:

$$X_{API}=a+b\times m, \quad (4)$$

where b is the slope of the linear relationship.

Following the SPhU approach for estimating linearity parameters in analytical procedure validation [11, 14], the shell's weight impact on X_{API} determination is insignificant if:

$$|a| \le \frac{\max\delta}{1-\left(\frac{X_{\min}}{100}\right)}, \quad (5)$$

where δ is the maximum permissible systematic error introduced by the shell's weight; $X_{\min}$ is the lower limit of the tablet weight range.

Note that the above formula assumes that the range is symmetrical, i.e., according to specification 100 %–$X_{\min}$=$X_{\max}$–100 % (i. e. less than 5 % according to the experimental data).

The contribution of $|a|$ in U_{NAP}^{tg} is considered insignificant if the following is satisfied:

$$\max\delta = 0.32\times U_{NAP}^{tg},$$

$$\max\delta = 0.32\times 0.96\,\% = 0.31\,\%. \quad (6)$$

Then the maximum allowable value for $|a|$ will make up:

$$|a_{\max}| = \frac{0.31}{1-95/100} = 6.1\,\%\left(\begin{matrix}\%\text{ relative to the weight}\\ \text{of the tablet core}\end{matrix}\right). \quad (7)$$

Thus, the coating weight's contribution is acceptable if it does not exceed 6.1 % of the tablet core weight under the condition that shell mass variation is significant.

4. 2. Selection of the object of analysis

The choice of the analysis object was guided by the objective of minimizing variations in results attributable to the specific nature of the object. Key considerations included:

1. Tablet mass homogeneity. It is essential that the tablet mass is homogeneous. The variation in the API content between tablets should predominantly be determined by the tablet's weight. Previous studies [24] have indicated that such homogeneity is typically observed when the API constitutes over 75 % of the tablet mass.

2. Complete API Release in Dissolution Test. The API should fully dissolve in the dissolution medium, ideally without necessitating modifications to the Dissolution test conditions. The API must be highly soluble in the dissolution medium for this to be probable.

3. Solution stability. The solutions analyzed must be stable enough so as not to introduce significant uncertainty during an extensive series of analyses.

Metformin film-coated tablets (500 mg dosage) produced by "Pharmex Group" LLC fulfil these criteria. The nominal content of Metformin HCl in the tablet mass is considerably high (92.3 %), surpassing the 75 % homogeneity benchmark. This implies expected homogeneity in API content, although this assumption requires experimental confirmation.

For these Metformin tablets, the nominal weights are 500.7 mg for the core and 9.8 mg for the film shell. Consequently, the shell-to-tablet weight ratio is approximately 1.8 %, considerably below the critical threshold of 6.1 % as per equation (7). This suggests that the tablet weight should be directly proportional to the API content.

The Dissolution and UDU tests for Metformin HCl are conducted using UV-spectrophotometry under as similar conditions as possible, adhering to the experimental planning guidelines outlined in Section 3. Both the test and reference solutions of Metformin HCl demonstrate long-term stability, confirmed over storage periods exceeding one month. According to the Ph. Eur. monograph [33], Metformin HCl is freely soluble in water, indicating an anticipated 100 % analyte release in the Dissolution test. Routine quality control results of Metformin tablets have confirmed this near-complete release.

In summary, the selected Metformin tablets meet all the stipulated criteria. However, the results from routine control exhibit high uncertainty levels, necessitating procedure optimization for accurate variability budget assessment.

4. 3. Optimization of Analytical Procedures

The analytical procedures for the Dissolution and UDU tests for Metformin tablets were validated by the SPhU approach to the uncertainty concept implementation [11], which is based on the Ph. Eur. and ICH recommendations [25]. Specifically, it was demonstrated that in a laboratory adhering to NAP [11, 25], the uncertainty of analysis results confidently (95 %) does not exceed U^{tg} of 3.0 % set for these tests (as per formula 1).

While these requirements are adequate for compliance testing of pharmaceutical products, the estimated uncertainty values obtained following the SPhU procedure exceeded the critical value of $U_{NAP}^{tg}=0.96\,\%$ (Tables 1, 2), which is crucial for checking the variability budget. To address this, we implemented several optimizations in our analytical procedures:

– weighing of individual tablets before analysis;

– increasing sample portions and the volumes in the volumetric flasks/pipettes;

– taking aliquots by weight.

The assessment of the combined uncertainty (NAP requirements) for these modified procedures was assessed as follows.

Following the SPhU approach, the laboratory must ensure that the actual uncertainty associated with standard analytical operations does not exceed the set critical value $\left(U_i^{tg}\right)$. These values correspond to the minimum acceptable uncertainty level for the laboratory involved in pharmaceutical product quality control, thus forming the NAP requirements. Therefore, these requirements must be met in any laboratory at any time.

The NAP requirements consider typical sources of variation characteristic of routine analyses. The U_i^{tg} values for volumetric glassware slightly exceed the ISO Class A requirements for bias. The NAP consideration includes the random uncertainty component introduced by the analyst during routine analysis.

Applying a block approach to uncertainty assessment [34] significantly simplifies the analysis of variation sources in the bottom-up assessment of result uncertainty. Thus, all sources of variation related to weighing are incorporated into the value U_i^{tg} recommended for weighing operations (0.2 mg for analytical balances). The laboratory bears the responsibility of qualifying the analytical balance and ensuring that the actual uncertainty for weighing operations, particularly for the range of weights used in UDU and Dissolution tests, remains within the defined target uncertainty U_i^{tg} of 0.2 mg.

Therefore, for standard analytical operations such as weighing, using volumetric glassware, and measurement, it is not necessary to dissect each component of variation of these standard operations. The primary objective is to demonstrate that the overall uncertainty in these operations does not exceed the NAP thresholds.

A thorough analysis of the calculation formula for the reportable result is typically sufficient to assess the combined uncertainty accurately. This approach negates the need for an in-depth analysis of the contributions of individual variation sources for standard analytical operations.

The content of Metformin HCl in mg/mg mass of an individual tablet, both for the UDU test (X_{UDU}) and the Dissolution test (X_{Diss}), is calculated using formulas derived from the optimized procedures outlined in Section 2:

$$X_{UDU} = \frac{A}{A_0} \times \frac{m_0}{m_i} \times \frac{m_{2ml}}{200 \times 200} \times \frac{500 \times 200}{2m_{2ml}} \times \frac{P}{100}, \quad (8),$$

$$X_{Diss} = \frac{A}{A_0} \times \frac{m_0}{m_i} \times \frac{m_{2ml}}{200 \times 200} \times \frac{900 \times 200}{m_{2ml}} \times \frac{P}{100}, \quad (9),$$

where A and A_0 are the average values of three absorbance measurements for the test solution and the reference solution, respectively; m_0, m_i and m_{2mL} are the weights of the reference standard (RS) portion, the tablet to be analyzed, and the 2 mL aliquot to be weighed (nominal values of 200 mg, 550 mg, and 2000 mg); 500 and 200 are the volumes (in mL) of the corresponding volumetric flasks; P is the content of metformin HCl in SPhU RS, in per cent; 900 is the volume of the dissolution medium measured by weight.

By the NAP recommendations set forth by the European Directorate for the Quality of Medicines & HealthCare (EDQM) [25] and the SPhU [10, 11], the impact of variation sources on the analytical procedure is estimated as follows.

The uncertainty associated with analytical weighing operations should not exceed 0.2 mg.

Then, the expanded weighing uncertainty, expressed as a 95 % one-sided confidence interval $\left(U_{balance}^{fact}\right)$, for the portions used in the analytical procedure, is calculated as follows:

$$U_{balance}^{fact} = \frac{0.2}{m} \times 100\ \%, \quad (10)$$

where m is the nominal value of the sample portion used in the procedure.

In the laboratory, a balance with a weighing uncertainty of 0.03 g is used for measuring the volume of the dissolution medium (900.0 mL). The uncertainty for measuring this volume is insignificant (0.003 %).

To estimate the uncertainty associated with the use of volumetric glassware in routine analysis (such as flasks and pipettes, denoted as $U_{vol,i}^{fact}$), we refer to the SPhU recommendations [11], as detailed in Tables 1 and 2.

For ISO class A volumetric flasks of 100 mL, 200 mL, and 500 mL, the target uncertainties according to NAP are 0.12 %, 0.1 %, and 0.07 %, respectively. These uncertainties are expressed as 95 % one-sided confidence intervals.

In line with SPhU's recommendations (2.2.25^{N}), the uncertainty for spectrophotometer measurements is estimated under the following conditions:

– a minimum of three measurements should be performed;

– the spectrophotometer must meet specific qualification requirements, including a maximum allowable relative standard deviation ($RSD_{\max}$) for optical density measurement results of 0.25 %;

– the expanded uncertainty for these measurements $\left(U_{progn}^{means}\right)$ is calculated as follows:

$$U_{means}^{fact} = \sqrt{2} \times \frac{1.64 \times 0.52}{\sqrt{3}} = 0.70\ \%, \quad (11)$$

where $\sqrt{2}$ is the coefficient that takes into account the variability in both the test solution and the reference solution (the variations in these two solutions are considered similar); 1.64 is the 95 % one-sided Student's t-coefficient for an infinite number of degrees of freedom; 0.52 is an $RSD_{\max}$ for optical density measurements as per the SPhU; 3 is the minimum number of measurements for each solution per the SPhU.

All sources of variation, which are understandable based on the calculation formula, are independent; the absence of covariance is assumed for them. Since all sources of variation are included in the calculation formula as products or quotients, the combined standard uncertainty is calculated as the square root from the sum of squares of standard uncertainties that represent estimations of the appropriate sources of variability, expressed as RSDs.

Following the SPhU approach, the same coefficient of coverage k is used to convert uncertainty estimates from all sources of variation expressed as intervals (expanded uncertainties) into standard uncertainty, and the same value of the coefficient k is used to convert the combined standard uncertainty into an expanded uncertainty, which should be expressed as a 95 % one-sided confidence interval. Therefore, the combined expanded uncertainty U_{NAP}^{fact} is estimated as follows:

$$U_{NAP}^{fact} = \sqrt{\sum \left(U_{NAP,i}^{tg}\right)^2}. \quad (12)$$

Tables 1, 2 present the uncertainty assessment results conducted for the original and optimized UDU and Dissolution procedures. The data clearly illustrate a notable reduction in the uncertainty levels for both the reference solution and the test solution, and the criterion set forth in equation (2) is satisfied for optimized procedures. The comparative analysis between the original and optimized procedures, as reflected in these tables, underscores the effectiveness of the optimization strategies.

The standard deviation for Metformin HCl content without accounting for individual tablet weights was 1.76 %. However, when considering the weight of individual tablets, this standard deviation is three times less – 0.61 %. Thus, the expanded uncertainty for the results of determining the API content in one tablet $\left(U_{UDU}^{\exp}\right)$ is:

$$U_{UDU}^{\exp} = RSD \times t\left(\text{one-sided}, n-1\right) = \\ = 0.61\,\% \times 1.6991 = 1.04\,\%. \quad (13)$$

Table 1

UDU analytical procedure optimization results – estimated value of uncertainty $U_{NAP}^{fact}\left(UDU\right)$ based on NAP requirements

Analytical procedure		Before optimization		After optimization	
		Analytical operation	Uncertainty, %	Analytical operation	Uncertainty, %
Reference solution	RS portion	100 mg	0.2	200 mg	0.1
	Flask	100 mL	0.12	200 mL	0.1
	Aliquot	1 mL	0.98	2 mL*	0.01
	Flask	100 mL	0.12	200 mL	0.1
Uncertainty of the reference solution:			**1.01**	–	**0.173**
Test solution	Weighing	1 tablet	Without weighing	1 tablet	0.055
	Flask	500 mL	0.07	500 mL	0.07
	Aliquot	1 mL	0.98	2 mLs*	0.01
	Flask	100 mL	0.12	200 mL	0.1
Uncertainty of the test solution:			**0.99**	–	**0.13**
U_{NAP}^{means}			**0.70**	–	**0.70**
Uncertainty for the SPhU RS			**0.5**	–	**0.5**
$U_{NAP}^{fact}\left(UDU\right)$			**1.80**	–	**0.88**

Note: aliquots were taken by weight

Table 2

The results of the optimization of the Dissolution analytical procedure – estimated value of uncertainty $U_{NAP}^{fact}\left(Diss\right)$ based on NAP requirements

Analytical procedure		Before optimization		After optimization	
		Analytical operation	Uncertainty, %	Analytical operation	Uncertainty, %
Reference solution	RS portion	55 mg	0.36	110 mg	0.18
	Flask	100 mL	0.12	200 mL	0.1
	Aliquot	1 mL	0.98	2 mL*	0.01
	Flask	100 mL	0.12	200 mL	0.1
Uncertainty of the reference solution			**1.06**	–	**0.23**
Test solution	1 tablet	Without weighing	–	1 tablet	0.055
	Aliquot	1 mL	0.98	2 mL*	0.01
	Flask	100 mL	0.12	200 mL	0.1
Uncertainty for the test solution			**0.99**	–	**0.11**
U_{NAP}^{fact}			**0.70**	–	**0.70**
Uncertainty for the SphU RS			**0.5**	–	**0.5**
$U_{NAP}^{fact}\left(Diss\right)$			**1.82**	–	**0.89**

Note: aliquots were taken by weight

4. 4. Compiling of the variability budget

Assessment of tablet mass homogeneity and average Metformin HCl content.

80 tablets were weighed, with the average tablet weight determined to be 0.5665 g. The RSD for the tablet weights was calculated at 1.71 %.

Based on the UDU test of 30 tablets, the average content of Metformin HCl was found to be 490.03 mg, i.e. within the specified range of 500±25 mg. This equates to 0.8670 mg of Metformin HCl per mg of tablet weight.

The similarity in standard deviations for tablet weight (1.71 %) and Metformin HCl content (1.76 %) suggests that the uncertainty of the optimized UDU analytical procedure practically has no impact on the result.

The substantial reduction in variation when adjusting for tablet mass (by a factor of 3) implies that the tablet mass is relatively homogeneous.

For the modified UDU procedure, all sources of variation are considered under control in the laboratory performing the analysis. This is supported by the fact that the experimental uncertainty estimate $\left(U_{UDU}^{\exp} = 1.04\,\%\right)$ is within 30 % of the bottom-up estimated uncertainty $\left(U_{NAP}^{fact} = 0.88\,\%\right)$, which is considered insignificant as per the requirements for assessing uncertainty at a 95 % confidence level ([10] for extended uncertainty, [32] for standard uncertainty).

Dissolution test results for variability budget compilation.

To control the completeness of the release (the "infinity point" condition), Dissolution test results were obtained for time intervals of 45 min and 60 min for the same solutions. This approach was based on the optimized analytical procedure. Increasing the basket rotation speed was considered unnecessary as the current speed was already set at an efficient rate of 100 rpm.

All results from the Dissolution test were calculated in terms of mg of Metformin HCl released per 1 mg of tablet weight. This calculation accounted for variability in the weight of each individual tablet.

The average results of Metformin HCl release determined in the Dissolution test are detailed in Table 3.

Table 3

Comparative results of Metformin HCl Release in the optimized dissolution test at 45 and 60 minutes

No of tablets	Metformin HCl release at 45 min intervals			Metformin HCl release at 60 min intervals		
	API content in mg/tablet	API content, % of the nominal value	API content in mg/mg tablet weight	API content in mg/tablet	API content, % of nominal value	API content in mg/mg tablet weight
1–6	–	–	–	490.00	100.41	0.8686
7–12	499.03	101.56	0.8786	501.42	102.05	0.8828
13–18	493.18	100.76	0.8716	492.65	100.65	0.8707
19–24	488.16	99.56	0.8612	488.53	99.63	0.8619
25–30	492.30	99.57	0.8651	492.67	99.65	0.8658
Mean	493.17	100.47	0.8691	493.05	100.57	0.8699
RSD	1.98	1.14	1.13	1.92	1.19	1.18

The Dissolution test results indicated that 100 % release of Metformin HCl was achieved within 45 min, i. e. there was no need for a volume correction for the 60-min measurement when calculating the results.

To evaluate the convergence of the variability budget, the combined estimated uncertainty (NAP requirements, Tables 1, 2) of the average results from both the Dissolution and UDU tests $\left(U_{comb}^{NAP}\right)$ was calculated by the formula:

$$U_{comb}^{NAP} = \sqrt{\frac{\left(U_{UDU}^{NAP}\right)^2}{n} + \frac{\left(U_{Diss}^{NAP}\right)^2}{m}}, \quad (14)$$

where n and m are the numbers of tablets used in the UDU and the Dissolution tests for compiling the variability budget.

For a Dissolution test release time of 45 min, U_{comb}^{NAP} was calculated at 0.47 %, based on testing 24 tablets for dissolution and 30 tablets for UDU tests. With a release time extended to 60 min, U_{comb}^{NAP} slightly decreased to 0.44 %, based on testing 30 tablets for both the Dissolution and UDU tests. Thus, these U_{comb}^{NAP} values demonstrate compliance with Criterion 2, which stipulates that the difference between UDU and Dissolution test results should not exceed 0.5 %.

The difference in the results of the UDU and Dissolution tests was calculated as follows:

– for 45 minutes:

(0.8691–0.8670)×100 %/0.8670=0.24 %;

– for 60 minutes:

(0.8699–0.8670)×100 %/0.8670=0.34 %.

The calculated differences for both the 45-min and 60-min intervals are well within the 0.5 % limit set by Criterion 2 and Criterion 1 (0.47 % and 0.44 %). This indicates the successful compilation of the variability budget for the 100 % release Dissolution test of the selected analyte in the laboratory that performed the analysis.

In compiling the variability budget, average values of the results were utilized. This approach confirms that all systematic sources of variation are effectively under control.

The conducted experiment allows for an in-depth assessment of the extent to which sources of variation affecting result precision are controlled. This is a crucial aspect, as it directly influences the reliability of decisions regarding compliance with specifications in routine analysis.

The estimated value of U_{comb}^{NAP} (Table 2) was calculated to be 0.89 %.

The expanded uncertainty based on experimental data is calculated as follows:

– for 45 minutes:

$$U_{Diss}^{\exp}(45\ \text{min}) = RSD(45\ \text{min}) \times t(n=24) =$$
$$= 1.13 \times 1.7139 = 1.94\ \%;$$

– for 60 minutes:

$$U_{Diss}^{\exp}(60\ \text{min}) = RSD(60\ \text{min}) \times t(n=30) =$$
$$= 1.18 \times 1.7109 = 2.0\ \%,$$

where t is the 95 % one-sided Student's coefficient.

The observed actual uncertainty values for both release times of the Dissolution test notably exceed the bottom-up estimated value (NAP) by more than 30 %. This deviation is not only statistically significant but also practically important.

When considering the target uncertainty (U^{tg}) for routine Dissolution analyses (as per formulas 1 and 2), the excess in actual uncertainty, approximately 1 %, surpasses the calculated threshold of $0.32 \times U^{tg}$(0.32×3.0 %=0.96 %).

Such a significant excess in actual uncertainty necessitates additional efforts. These efforts should aim to identify and bring into control the unknown source(s) of variation contributing to this discrepancy.

Despite this excess, the actual uncertainty value still falls within the acceptable limits of U^{tg} for Dissolution test results as recommended by the SPhU (formula 1). Hence, the analytical procedure remains valid for use in routine quality control of the Dissolution test.

Study limitations and prospects for further research. The proposed suitability criteria are stringent, aligning more with calibration tasks (such as RS certification or volumetric glassware verification) than routine analysis tasks. It is advisable to apply the variability budget approach to the Dissolution test for another analysis object in a different laboratory to validate the feasibility of these criteria.

An important conclusion of the conducted research is identifying an unknown source of variation in the Dissolution test, which is statistically and practically significant for routine analysis tasks but not observed in the

UDU test. Repeating the study with a different SDU in another laboratory is recommended to investigate and bring this source under control.

The absence of suitable uncoated tablet options limited the study. The film coating on Metformin tablets introduces an additional, indirectly controlled variation source. Although the UDU test results support the theoretical insignificance of shell weight variation, the presence of an unknown variation source suggests the need to repeat the study on tablets without shells.

In pharmaceutical standardization, analytical variation characteristic of NAP (routine practice) is already considered in criteria set by pharmacopoeias. A laboratory must ensure that variation from standard analytical operations does not exceed NAP levels $\left(U_i^{tg}\right)$, and that the reportable result's uncertainty stays within the U^{tg} for the analytical task. However, as professional testing results show, the actual uncertainty in volumetric glassware operations can significantly exceed U_i^{tg} if not explicitly controlled [35, 36].

The analytical procedures for Dissolution and UDU were modified to reduce or control standard variation sources. However, certain operations, such as using a 200 mL volumetric flask and weighing the test portion, remained outside strict experimental control. Further development and implementation of personnel qualification procedures to meet NAP requirements are necessary. After introducing these procedures, the study should be repeated for the Dissolution test's variability budget.

The approaches developed in this study have multiple potential applications:

– as a standardized procedure for pharmaceutical development of the Dissolution test where 100 % release of the active substance is achievable;

– as a basis for transferring the analytical procedure of the Dissolution test, particularly for preparations ensuring 100 % release;

– for continued procedure performance verification in routine analysis during the production of studied Metformin 500 mg tablets;

– as a method for certifying test items for the Dissolution test in intra- and inter-laboratory quality control of analysis results.

5. Conclusion

This study marks the first instance of controlling variation sources for a 100 % release Dissolution test by compiling a variability budget using the State Pharmacopoeia of Ukraine (SPhU) metrological approach. We proposed metrological criteria and an experimental design tailored for this purpose. Uniquely within pharmaceutical development, we employed a technique not for routine quality control but specifically adapted to study sources of variation and assess their impact on the analysis results. This approach represents a significant advancement in analytical method development.

For the UDU test, it was demonstrated that the laboratory effectively controls all significant sources of variability at the level of normal analytical practice. In the 100 % release Dissolution test, all sources of variation affecting the assay results are also under control. However, there is a need for additional research to identify and manage an unknown source of practically significant random variation.

The findings illustrate the feasibility of the proposed approach, including the experimental design and criteria. These include the transfer of the Dissolution test, continued procedure performance verification in routine analysis, and the certification of test items for both intra- and inter-laboratory quality control of analysis results.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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Dmytro Leontiev, Doctor of Pharmaceutical Sciences, Senior Researcher, Head of Department, Department of Validation and Reference Standards, State Enterprise "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicines", Astronomichna str., 33 Kharkiv, Ukraine, 61085, Professor, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Vitalii Asmolov, Postgraduate Student, Department of Pharmaceutical Chemistry, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002, Quality Control Scientist, Noven Pharmaceuticals, Inc., 11960 SW 144th str., Miami, USA, 33186

Natalia Volovyk*, PhD, Senior Researcher, Deputy Director for Quality, State Enterprise "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicines", Astronomichna str., 33, Kharkiv, Ukraine, 61085

Oleksandr Gryzodub, Doctor of Chemical Sciences, Professor, Chief Researcher, Department of State Pharmacopoeia of Ukraine, State Enterprise "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicines", Astronomichna str., 33, Kharkiv, Ukraine, 61085

***Corresponding author:** *Natalia Volovyk, e-mail: nvolovyk_rs@ukr.net*