UNIVERSIDADE DE SÃO PAULO

Faculdade de Ciências Farmacêuticas

Programa de Pós-Graduação em Farmácia (Fármacos e Medicamentos)

Área de Produção e Controle Farmacêuticos

Avaliação da incerteza em ensaios de dissolução de comprimidos de prednisona e glibenclamida disponíveis no mercado

Adriano Yuuki Sano

Dissertação para obtenção de Título de Mestre

Orientador: Prof. Dr. Felipe Rabelo Lourenço

São Paulo

2023

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Versão Original

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“Dedico este trabalho aos meus pais que me apoiaram em toda minha trajetória acadêmica.”

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1. Teste de Dissolução. 2. Incerteza de Medição. 3. Avaliação de Conformidade. 4. Risco de decisões falsas. I. T. II. Lourenço, Felipe Rebello, orientador.

SUMÁRIO

RESUMO

SANO, A.Y. **Avaliação da incerteza em ensaios de dissolução de comprimidos de prednisona e glibenclamida disponíveis no mercado.** 2023. 65p. Dissertação (Mestrado) – Departamento de Farmácia, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2023.

O teste de dissolução é um procedimento de extrema importância para avaliar a performance de formas farmacêuticas sólidas *in-vitro*. Ele desempenha um papel essencial no controle de qualidade e em estudos de pré-formulação. Considerando a importância dos resultados desse teste para determinar se um medicamento está ou não em conformidade, torna-se fundamental avaliar a qualidade desses resultados. O objetivo deste trabalho é avaliar as incertezas de medição associadas aos resultados analíticos obtidos nos testes de dissolução de comprimidos contendo prednisona e glibenclamida, que são fármacos classificados, respectivamente, como classe I e classe II no Sistema de Classificação Biofarmacêutica. O valor de incerteza encontrado para o teste de dissolução para comprimidos de prednisona foi de 2,2%, abaixo do valor alvo de incerteza (u^t = 2,5%). A contribuição das fontes de incerteza foi de 24% para a amostragem, 29% para a etapa de dissolução e 47% para a etapa de quantificação. Após a realização de análise de risco, o lote de comprimidos de prednisona foi considerado conforme, com um risco total reduzido de decisão incorreta (valor de risco total abaixo de 5%). A incerteza do teste de dissolução de comprimidos de glibenclamida foi de 6,33%. As fontes de incerteza que contribuíram para a incerteza geral de medição do teste de dissolução foram estimadas em 76,09% para a amostragem, 22,15% para a etapa de dissolução e 1,76% para a etapa de quantificação. Os resultados demonstram que o teste de dissolução dos comprimidos de glibenclamida apresentou uma alta incerteza de amostragem. O lote de comprimidos de glibenclamida não passou no teste de dissolução (dissolução abaixo dos limites especificados), provavelmente devido à baixa solubilidade da glibenclamida. O estudo enfatiza a importância de avaliar a incerteza de medição nos testes de dissolução para garantir um controle de qualidade confiável e preciso dos produtos farmacêuticos.

PALAVRAS-CHAVE: Teste de Dissolução, Incerteza de Medição, Avaliação de Conformidade, Risco de decisões falsas

ABSTRACT

SANO, A.Y. **Evaluation of uncertainty in dissolution tests of prednisone and glyburide tablets available in the market.** 2023. 65p. Master's Degree - Pharmacy Department, Faculty of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2023.

Dissolution testing is an extremely important procedure for evaluating the in vitro performance of solid pharmaceutical dosage forms. It plays an essential role in quality control and pre-formulation studies. Considering the significance of the test results in determining whether a medication is compliant or not, it becomes crucial to assess the quality of these results. The aim of this study is to evaluate the measurement uncertainties associated with the analytical results obtained in the dissolution tests of prednisone and glibenclamide tablets, which are classified as Class I and Class II drugs, respectively, in the Biopharmaceutical Classification System. The uncertainty value for the dissolution test of prednisone tablets was found to be 2.2%, below the target uncertainty value (u^t = 2.5%). The contributions of uncertainty sources were 24% from sampling, 29% from the dissolution step, and 47% from the quantification step. After conducting a risk analysis, the batch of prednisone tablets was deemed compliant, with a reduced total risk of false decision (total risk value below 5%). The uncertainty of the dissolution test for glibenclamide tablets was 6.33%. The sources of uncertainty contributing to the overall measurement uncertainty of the dissolution test were estimated to be 76.09% from sampling, 22.15% from the dissolution step, and 1.76% from the quantification step. The results demonstrate that the dissolution test of glibenclamide tablets exhibited high sampling uncertainty. The batch of glibenclamide tablets did not pass the dissolution test (dissolution below the specified limits), likely due to the low solubility of glibenclamide. This study emphasizes the importance of evaluating the measurement uncertainty in dissolution testing to ensure reliable and accurate quality control of pharmaceutical products.

KEYWORDS: Dissolution Test, Measurements Uncertainty, Compliance Assessment, Risk of False Decisions

LISTA DE QUADROS E TABELAS

LISTA DE FIGURAS

LISTA DE SIGLAS E ABREVIATURAS

SCB ou BCS – Sistema de Classificação Biofarmacêutica ou Biopharmaceutical Classification System

USP – United States Pharmacopeia

EMA – European Medicines Agency

RENAME – Relação Nacional de Medicamentos Essenciais

UV – Ultravioleta

DoE – Design de Experimentos (Design of Experiments)

u^t – Incerteza alvo (Target Uncertainty)

Q – Limite de especificação

ICH – Conferência Internacional de Harmonização (Internacional Conference on Harmonization)

FDA – Food and Drug Administration

RPM – Rotações por minuto

CRS – Certified Reference Substance

Eq. – Equação

$u_{\%}$ – Incerteza combinada

u_s – Incerteza da amostragem

u_d – Incerteza da etapa de dissolução

u_q – Incerteza da etapa de quantificação

$U_{\%}$ – Incerteza expandida

k – Fator de abrangência

g – Guarda de banda univariada

g' – Guarda de banda multivariada

A – Limite de aceitação

ANOVA – Análise de variância

d.f. – graus de liberdade

SS – Soma dos quadrados

MS – Quadrado da média

IFA ou API – Ingrediente farmacêutico ativo ou active pharmaceutical ingredient

INTRODUÇÃO

1. INTRODUÇÃO

1.1. O Teste de Dissolução

O teste de dissolução é um procedimento essencial para avaliar a taxa e extensão com que o fármaco presente numa forma farmacêutica sólida, como comprimidos ou cápsulas, se dissolverá em um meio de dissolução (FARMACOPEIA BRASILEIRA, 2019). O teste é amplamente utilizado na indústria farmacêutica para verificar a qualidade, desempenho e consistência de formulações sólidas. Além disso, o teste de dissolução desempenha um papel crucial na avaliação da biodisponibilidade e bioequivalência de medicamentos genéricos em relação aos produtos de referência (SHAH, 2001).

A importância do teste de dissolução reside na sua capacidade de fornecer informações valiosas sobre a taxa de liberação de um fármaco a partir de uma forma farmacêutica sólida, permitindo a previsão do seu desempenho *in vivo* (STORPIRTIS et al., 2004). Através da avaliação da dissolução, é possível identificar lotes fora de especificação, detectar possíveis problemas de formulação, comparar diferentes produtos e otimizar as formulações farmacêuticas. Além disso, o teste de dissolução desempenha um papel fundamental no desenvolvimento de medicamentos, permitindo o aprimoramento de formulações e o cumprimento das regulamentações estabelecidas pelos órgãos regulatórios (FARMACOPEIA BRASILEIRA, 2019; USP, 2021). Em resumo, o teste de dissolução desempenha um papel crítico na garantia da qualidade, segurança e eficácia dos medicamentos sólidos, sendo uma ferramenta essencial para a indústria farmacêutica (SHAH, 2001).

1.2. O Sistema de Classificação Biofarmacêutica

O sistema de classificação biofarmacêutica (SCB) é uma ferramenta desenvolvida para prever o desempenho biológico de um medicamento com base em suas características físico-químicas e propriedades de dissolução. Esse sistema classifica os fármacos em quatro categorias (SCB I, II, III e IV), levando em consideração a solubilidade e permeabilidade do fármaco (**Figura 1**). A importância do SCB reside na sua capacidade de fornecer informações cruciais para o desenvolvimento e registro de medicamentos, auxiliando na formulação e avaliação da biodisponibilidade e bioequivalência (AMIDON et al., 1995).

Figure 1. Classes do SCB e suas respectivas características de solubilidade e permeabilidade

O desenvolvimento do SCB foi um marco importante na indústria farmacêutica, buscando estabelecer correlações entre a dissolução do fármaco e sua absorção no organismo. Ele foi proposto pela primeira vez em 1995 por Amidon e colaboradores e tem sido amplamente aceito e adotado internacionalmente (EMA, 2020a).

De acordo com as diretrizes regulatórias, os medicamentos de Classe I podem se beneficiar de bioisenções, que são aprovações regulatórias para evitar estudos *in vivo* de biodisponibilidade e bioequivalência. Para isso, é necessário demonstrar que o fármaco atende aos critérios estabelecidos de dissolução *in vitro* (EMA, 2020b, 2020a).

Para os medicamentos da Classe II, que possuem baixa solubilidade e alta permeabilidade, o teste de dissolução é fundamental para avaliar a qualidade do medicamento, uma vez que a dissolução é o processo limitante para a biodisponibilidade do fármaco. Essa informação é crucial para prever a velocidade e a extensão da absorção do fármaco no organismo. Ao avaliar a dissolução do medicamento, é possível identificar e otimizar formulações que promovam uma dissolução adequada e, assim, melhorem a biodisponibilidade do fármaco (STORPIRTIS et al., 2004).

1.3. Avaliação da Incerteza

A avaliação da qualidade dos resultados do teste de dissolução é de extrema importância para a determinação da conformidade ou não-conformidade de um medicamento. É essencial garantir a confiabilidade e a precisão dos dados analíticos, uma vez que resultados insuficientes podem acarretar consequências desastrosas. Nesse contexto, a incerteza de medição desempenha um papel fundamental, definindo a dispersão dos valores que podem ser razoavelmente atribuídos a um resultado. A avaliação da incerteza de medição envolve a identificação e quantificação das fontes de incerteza, a conversão dos componentes de incerteza em incerteza padrão e o cálculo

das incertezas combinada e expandida (BETTENCOURT DA SILVA; WILLIAMS, 2015; TRAPLE et al., 2014).

Existem duas abordagens comumente utilizadas para estimar a incerteza combinada: a abordagem *bottom-up* e a abordagem *top-down*. A abordagem *bottom-up* é mais trabalhosa, exigindo uma quantidade maior de informações, mas permite identificar as fontes de incerteza mais significativas e auxiliar no controle e na redução da incerteza final. Métodos como a lei de propagação de incertezas, a planilha de Kragten e a simulação de Monte Carlo são utilizados nessa abordagem. Por outro lado, a abordagem *top-down* é menos trabalhosa, porém não permite quantificar individualmente a contribuição de cada fonte de incerteza, geralmente resultando em uma incerteza final superestimada. Métodos como a incerteza a partir dos resultados de validação de métodos e estudos de repetibilidade e reprodutibilidade são utilizados nessa abordagem (ROMERO; LOURENÇO, 2017; SEPAROVIC et al., 2023).

Considerando que os valores medidos estão sempre associados a uma medida de incerteza, riscos de decisões falsas quanto à conformidade ou não-conformidade do medicamento podem surgir. Portanto, é necessário aplicar regiões de aceitação/rejeição (*guard-bands*) que levem em consideração a incerteza de medição, a fim de minimizar esses riscos (DA SILVA; LOURENÇO, 2023). A partir da incerteza de medição, é possível avaliar diferentes tipos de riscos, como os riscos do consumidor e do produtor, riscos globais e específicos, riscos particulares e riscos totais. O risco do consumidor refere-se à probabilidade de aceitar erroneamente um lote de má qualidade, enquanto o risco do produtor é a probabilidade de rejeitar um lote de boa qualidade. O cálculo do risco de falsa decisão de conformidade é relevante, especialmente quando os resultados da medição estão próximos dos limites de especificação e/ou quando o valor de incerteza é elevado (BETTENCOURT DA SILVA et al., 2019; DE OLIVEIRA;

LOURENÇO, 2021; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2023). A avaliação da qualidade dos resultados do teste de dissolução e a consideração da incerteza de medição são essenciais para garantir a conformidade dos medicamentos. A correta estimativa da incerteza e a aplicação de regiões de aceitação/rejeição ajudam a minimizar os riscos de decisões falsas, garantindo a segurança e a eficácia dos produtos farmacêuticos (KUSELMAN et al., 2017a).

JUSTIFICATIVA

2. JUSTIFICATIVA

A avaliação da incerteza de medição possui grande importância para a tomada de decisões de conformidade ou não conformidade de produtos farmacêuticos, no entanto são poucos os estudos de incerteza para o método de dissolução.

Desta forma foram selecionados dois fármacos, sendo um deles da classe I do SCB e o outro da classe II. Medicamentos destas classes foram escolhidos pois a avaliação da conformidade nestes casos é mais crítica, uma vez que medicamentos da classe I são candidatos a bioisenções, enquanto os de classe II possuem como etapa limitante para a biodisponibilidade o processo de dissolução.

Como representante dos fármacos de classe I foi selecionada a prednisona, uma vez que os comprimidos de prednisona (usualmente fornecidos pela farmacopeia americana – USP) são utilizados para a calibração de dissolutores, permitindo assim uma melhor rastreabilidade metrológica do estudo. Já para a classe II, foi selecionada a glibenclamida, um fármaco hipoglicemiante de uso contínuo amplamente utilizado e constante na Relação Nacional de Medicamentos Essenciais (RENAME).

OBJETIVOS

3. OBJETIVOS

3.1. Objetivos Gerais

O objetivo do presente projeto é avaliar as incertezas de medição associadas ao teste de dissolução de comprimidos de prednisona e de glibenclamida e, desta forma, estimar o risco de decisões falsas quanto a aprovação ou reprovação de um lote de medicamento.

3.2. Objetivos Específicos

- Identificar as fontes de incerteza relacionadas ao teste de dissolução;
- Avaliar a incerteza associada às etapas de amostragem, dissolução e quantificação pelas abordagens *top-down* e/ou *bottom-up;*
- Obter a incerteza combinada e expandida, calculadas a partir das fontes de incerteza relevantes;
- Estimar os riscos de decisões falsas quanto à conformidade / não-conformidade, devido à incerteza de medição;
- Empregar a informação da incerteza de medição na avaliação de conformidade de medicamentos.

CAPÍTULO I

(CHAPTER I)

4. CHAPTER I - MEASUREMENT UNCERTAINTY ARISING FROM SAMPLING AND ANALYTICAL STEPS OF DISSOLUTION TEST OF PREDNISONE TABLETS

ABSTRACT

Dissolution is used to determine the rate and extent of drug release from the dosage form into a dissolution medium, which allow to assess the batch-to-batch variability. Considering that the dissolution test is used to predict the vivo performance of the drug as well, it is important to guarantee the quality and reliability of dissolution test results. The aim of this work was to evaluate the measurement uncertainty arising from sampling and analytical steps of dissolution test of prednisone tablets. Dissolution test was performed using 900 mL of purified water as dissolution medium and a dissolution apparatus equipped with paddles rotating at 50 rpm for 30 minutes. Quantification was performed by UV spectrophotometer. Uncertainty arising from sampling was estimated using the duplicate method (empirical approach), using 17-sampling target, two samples for each sampling target, and three replicas for each sample, totalizing 102 analyses. Uncertainty arising from analytical steps considered the uncertainty from dissolution step (estimated using Monte Carlo method and regression equation obtained using DoE) and uncertainty from quantification step. Overall uncertainty value was found to be 2.2%, which is below the target uncertainty value (u^t =2.5%). The contributions of uncertainty sources in this study were as follows: 24% from sampling uncertainty, 29% from the dissolution step uncertainty, and 47% from the quantification step uncertainty. The results of dissolution test should be compared to the specification limits (Q). According to the pharmacopeia requirements, the batch of the medicine should be declared compliant if the dissolved amount of prednisone for six tablets are above the specification limits +5% (Q+5%=85%). Since the measured values for all six tablets (96.5%, 94.0%, 96,4%, 95.3%, 96.0%, and 96.9%) were above the multivariate acceptance limit (90.2%, calculate as the standard uncertainty multiplied by multivariate coverage factor), the batch of the prednisone tablets was declared complaint, with a reduced total risk of false decision (total risk value below 5%).

KEYWORDS: Dissolution test; measurement uncertainty; conformity assessment

4.1. Introduction

Dissolution testing is a critical step in the development and quality control of drugs. The dissolution test is used to determine the rate and extent of drug release from the dosage form into a dissolution medium. The results of the dissolution test are used to predict the *in vivo* performance of the drug and to ensure batch-to-batch consistency. In addition, the dissolution test works as a bioequivalence signal, being used to identify bioavailability problems, and assess the need for bioequivalence tests (SHAH, 2001).

In the 1900s, Amidon and collaborators introduced the Biopharmaceutical Classification System (BCS) being based on the fundamental properties that govern the absorption of drugs (AMIDON et al., 1995). The BCS is a classification system that categorizes drugs based on their solubility and permeability characteristics. This classification system is used to predict the oral bioavailability of a drug, which is a measure of how much of the drug is absorbed into the bloodstream after oral administration (AMIDON et al., 1995; DOKOUMETZIDIS; MACHERAS, 2006). The BCS categorizes drugs into four classes: Class I (high solubility, high permeability), Class II (low solubility, high permeability), Class III (high solubility, low permeability), and Class IV (low solubility, low permeability) (AMIDON et al., 1995; SHAH, 2001).

The BCS is a useful tool for drug development, as it provides a framework for understanding the factors that influence the oral bioavailability of a drug and for identifying formulation strategies to improve bioavailability. It is also useful for predicting the potential for drug-drug interactions, as drugs with high oral bioavailability are more likely to cause interactions with other drugs (ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL

REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2015, 2017, 2023).

Since the introduction of the BCS, Regulatory Agencies have established regulations and guidelines (EMA, 2020b, 2020a; FDA, 2017) acknowledging the suitability of BCS Class I drugs for biowaiver, emphasizing the significance of *in vitro* dissolution testing as an essential test to establish the pharmaceutical equivalence of a generic medicine and guarantee it's quality. Therefore, the main objective of the BCS is to estimate the pharmacokinetic profile of a drug from permeability and solubility data, in order to identify cases in which the exemption of bioequivalence studies is possible (DOKOUMETZIDIS; MACHERAS, 2006; FDA, 2017).

The BCS Class I drugs have high solubility in aqueous media and high permeability across biological membranes. They are rapidly absorbed into the bloodstream and have high oral bioavailability. Examples of Class I drugs include aspirin, ibuprofen and prednisone (AMIDON et al., 1995; DOKOUMETZIDIS; MACHERAS, 2006; SHAH, 2001). Prednisone tablets were selected for this work as it is often used during chemical qualification of dissolution apparatus, according to the United States Pharmacopeia (USP, 2021). For BCS Class I drugs, a simple dissolution test with a single medium, such as pH 6.8 buffer, is usually sufficient to assess the performance of the drug. These drugs have high solubility and high permeability, so they dissolve rapidly and are rapidly absorbed into the bloodstream. The dissolution test for Class I drugs is typically performed at a low stirring speed (50-100 RPM) to simulate the conditions in the gastrointestinal tract (EUROPEAN PHARMACOPEIA (PH. EUR.), 2020; FARMACOPEIA BRASILEIRA, 2019; USP, 2021).

The results of the dissolution test should be compared to a set of acceptance criteria, such as pharmacopeial standards or in-house specifications, to ensure that the

drug meets the desired quality standards. The dissolution test should be performed on a representative sample of the drug product to ensure that the results are representative of the entire batch (EUROPEAN PHARMACOPEIA (PH. EUR.), 2020; FARMACOPEIA BRASILEIRA, 2019; USP, 2021).

Measurement uncertainty is typically expressed as a range of values that reflects the degree of confidence or probability that the true value of a measured quantity lies within that range (ELLISON; WILLIAMS, 2012; RAMPSEY; ELLISON; ROSTRON, 2019). This range is determined through statistical analysis and can be used to evaluate the reliability of the measurement result. Managing and reducing measurement uncertainty is important in medical analysis, as it can impact the interpretation of results and the decisions made based on those results (ELLISON; WILLIAMS, 2012; RAMPSEY; ELLISON; ROSTRON, 2019).

Measurement uncertainty in medical analysis can arise from both the sampling and analytical steps of the measurement process (SEPAROVIC et al., 2023; TRAPLE et al., 2014). Uncertainty in the sampling step may arise from factors such as the heterogeneity of the sample, the sampling method used, and the sample preparation process. For example, if a small or non-representative sample is taken, this can introduce uncertainty into the measurement results. Uncertainty in the analytical step may arise from factors such as instrument calibration, measurement errors, methodological variations, environmental conditions, among other sources. For example, if an instrument is not calibrated correctly, or the measurement is affected by environmental factors such as temperature or humidity, this can introduce uncertainty into the measurement results (ELLISON; WILLIAMS, 2012; RAMPSEY; ELLISON; ROSTRON, 2019; SEPAROVIC et al., 2023; TRAPLE et al., 2014).

The combined uncertainty can be obtained from the individual standard uncertainties using bottom-up or top-down approaches. In the bottom-up approach, the systematic and random errors of each step of the analytical procedure are combined to obtain the overall measurement uncertainty, while in the top-down approach, the systematic and random errors of the analytical procedure as a whole are combined to obtain the measurement uncertainty (ELLISON; WILLIAMS, 2012; RAMPSEY; ELLISON; ROSTRON, 2019; SEPAROVIC et al., 2023; TRAPLE et al., 2014).

Considering measurement uncertainty information, it is possible to evaluate different types of risks, such as consumer's and producer's risks, global and specific risks, particular and total risks. Consumer's risk is defined as the risk of incorrectly accepting a poor-quality lot (with a result outside the specification limits), while producer's risk is defined as the risk of rejecting a good quality lot (with a result within the specification limits) (BETTENCOURT DA SILVA et al., 2019; BETTENCOURT DA SILVA; LOURENÇO; HIBBERT, 2022; KUSELMAN et al., 2017a, 2017b; PENNECCHI et al., 2018).

The risk of a false compliance decision can be negligible when the measured value is in the target specification range. However, if the measured value is close to the upper or lower limits, the risk of false decision is relevant and must be calculated (JCGM 106:2012, 2022; WILLIAMS et al., 2021).

Alternatively, guard-bands can be adopted to minimize the risk of false conformity decisions in medicine analysis. Guard-bands are added or subtracted to the specification limits in order to provide an additional margin of safety or assurance. By changing the acceptable range, the probability of false conformity decisions is reduced (JCGM 106:2012, 2022; WILLIAMS et al., 2021). This is particularly important when there is a risk of harm to human health or safety if a product fails to meet the required

standards. Guard-bands are typically established based on the level of risk involved (adopting an appropriate coverage factor) and the available knowledge about the measurement results (measured value and its uncertainty) (DA SILVA; LOURENÇO, 2023; DE OLIVEIRA; LOURENÇO, 2021).

Conformity assessment of *in vitro* dissolution test results is a relevant issue for pharmaceutical industries, particularly for BCS class I drug which will not be subject to bioequivalence test (biowaiver). Since the approval of these medicines are only based on *in vitro* dissolution test, it is important to take into account the measurement uncertainty information for conformity assessment. To the best of our knowledge, we did not find in the literature works that provide a detail measurement uncertainty evaluation for dissolution test.

The aim of this work is to evaluate the measurement uncertainty arising from sampling and analytical steps of dissolution test of prednisone tablets. In addition, measurement uncertainty was used to establish guard-bands to ensure an increased probability of correct acceptance (in other words, a reduced consumer's risk).

4.2. Materials and Methods

4.2.1. Dissolution test of prednisone tablets

Prednisone tablets samples were purchased in Brazilian marked. Prednisone certified reference substance (CRS) was obtained from United States Pharmacopoeia (USP) (USP, 2021).

Dissolution test was performed using 900 mL of purified water as dilution medium, using a dissolution apparatus (Nova Ética, 299) equipped with paddles rotating at 50 rpm. After 30 minutes, 5-10 mL aliquots of dissolution medium were withdrawn and properly diluted using purified water as diluent. Prednisone quantification was performed using a UV spectrophotometer (Thermo, Genesys 50) adjusted to 242 nm and a 1-cm quartz cuvette. The amount of prednisone dissolved for each tablet was calculated by comparing the absorbances of sample solutions to the absorbances of prednisone CRS solutions at concentrations from 10 to 30 μg/mL (FARMACOPEIA BRASILEIRA, 2019). The UV spectrophotometric method was adopted in this work, since it is the official method in both Brazilian pharmacopeia and United States Pharmacopeia (FARMACOPEIA BRASILEIRA, 2019; USP, 2021).

According to the pharmacopeial requirements, the amount of prednisone dissolved for each of six tablets must be not less than 85% (Q = 80%, in stage 1 all dissolved amount for all tablets must be not less than Q+5%) of the label content (FARMACOPEIA BRASILEIRA, 2019; USP, 2021).

4.2.2. Measurement uncertainty evaluation

Measurement uncertainty evaluation involves four steps: definition of measurand; identification of measurement uncertainty sources; quantification of measurement components and conversion to standard uncertainties; and calculation of combined and expanded uncertainties.

The measurand was defined as the amount of prednisone dissolved after prednisone tablets had been subjected to dissolution test, according to pharmacopeial

requirements (COOK; ADDICKS; WU, 2008; EMA, 2020b). The sources of uncertainty were identified and systematically listed in a cause-effect (Ishikawa diagram), showed in **Figure 2**. Quantification of measurement components and conversion to standard uncertainties are provided in sections 4.2.2.1 and 4.2.2.2, and calculation of combined and expanded uncertainties are provided in section 4.2.2.3.

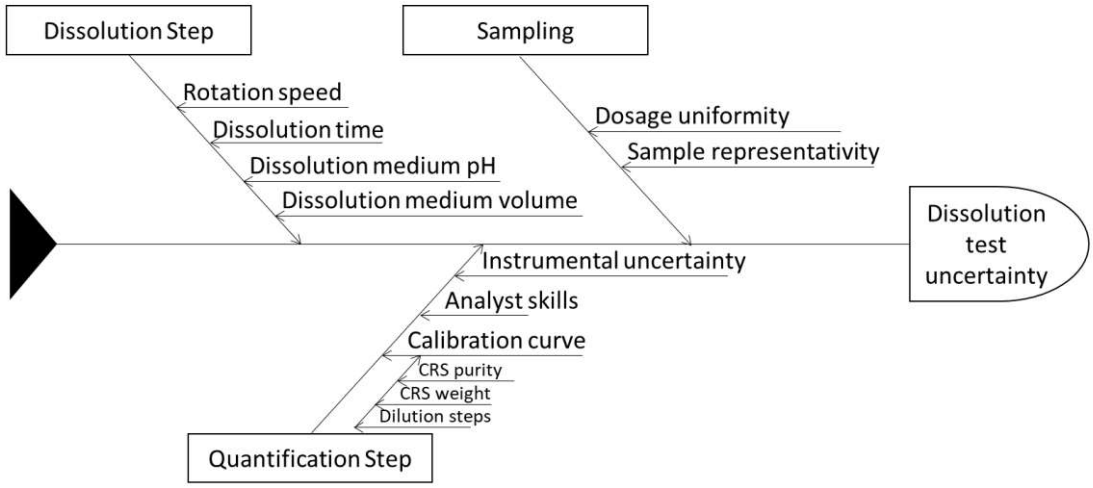

Figure 2. Cause-effect (Ishikawa) diagram with the sources of uncertainty related to the dissolution test of prednisone tablets.

4.2.2.1. Uncertainty arising from sampling

Uncertainty arising from sampling was estimated using the duplicate method (empirical approach), which is a simple and most cost-effective method. In the duplicate method, 17-sampling target were subject to dissolution test. For each of the 17-sampling target, two samples were collected, resulting in 34 samples. All 34 samples were analyzed three times (triplicates), resulting in 102 analyses. The random component of the uncertainty arising from sampling was estimated by applying analysis of variance

(ANOVA) to the measurements of the amount of prednisone dissolved on the duplicated samples (RAMPSEY; ELLISON; ROSTRON, 2019).

4.2.2.2. Uncertainty arising from analytical steps

Uncertainty arising from analytical steps considered two main sources of uncertainty: 1) uncertainty from dissolution step; and 2) uncertainty from quantification step. Both steps were studied in detail as described below.

The measurement uncertainty from dissolution step was performed Monte Carlo method in association with a regression model that explains the amount of prednisone dissolved as function of paddle rotation speed (X_1, from 40 to 60 rpm), volume of dissolution medium (X_2, from 800 to 1000 mL), time of dissolution (X_3, from 20 to 40 minutes), and pH of dissolution medium (X_4, from 4.0 to 9.6). The regression model was obtained from experimental data, using a factorial design (ROMERO; LOURENÇO, 2017; SAVIANO; LOURENÇO, 2018).

The evaluation of uncertainties arising from the quantification step considered the uncertainty from linear least squares calibration [14], using **Eq. (1)**:

$$u_q = \frac{s}{b}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(q-\bar{c})^2}{\sum_i^n (c_i-\bar{c})^2}} \qquad \text{Eq. (1)}$$

Where, c_0 and u_{c_0} is the concentration of prednisone in the sample solution and its respective uncertainty, s is the residual standard deviation, b is the slope of least square regression, p and n are the number of measurements for sample and standard

solutions, c_i is the concentration of the calibration standard corresponding to the ith measurement (n number of measurements), and $\bar{c}$ is the mean value of the calibration standards (n number of measurements).

4.2.2.3. Combined measurement uncertainty

The combined measurement uncertainty was obtained using the law of uncertainty propagation (ELLISON; WILLIAMS, 2012; SEPAROVIC et al., 2023), using **Eq. (2)**.

$$u_{\%} = \sqrt{{u_s}^2 + {u_d}^2 + {u_q}^2} \qquad \text{Eq. (2)}$$

Where, $u_{\%}$ is the combined uncertainty of the dissolution test for prednisone tablets, u_s is the uncertainty arising from sampling, u_d is the uncertainty from dissolution step, and u_q is the uncertainty from quantification step.

The expanded uncertainty ($U_{\%}$) was obtained by multiplying the combined uncertainty ($u_{\%}$) by an appropriate coverage factor (k). Typically, k = 2 is used for an approximately 95% confidence level.

4.2.3. Conformity assessment

Univariate (g) and multivariate (g') guard-band widths were calculated by multiplying the combined measurement uncertainty by appropriate coverage factors (k

and k' for univariate and multivariate guard bands, respectively). Coverage factor values used were 1.64 and 2.38 for univariate and multivariate guard bands, respectively, for an approximately 95% confidence level (DE OLIVEIRA; LOURENÇO, 2021; ROMERO; LOURENÇO, 2017).

The guard-band widths (g and g') were summed up to the specification limit (Q+5% = 85% for stage 1) to obtained univariate and multivariate acceptance limits (A = 85% + g and A' = 85% + g') [25,26]. The product should be declared compliant if the dissolved amount of prednisone for all six tablets are above the multivariate acceptance limits. In this case, the total risk of false decisions should be less than 5%.

4.3. Results and Discussion

4.3.1. Uncertainty arising from sampling and analytical steps

Uncertainty arising from sampling was evaluated by analyzing 17-sampling target to dissolution test. For each of 17-sampling target, two samples were collected; and all 34 samples were analyzed three times (triplicates, resulting in 102 analysis. The ANOVA results indicate that the uncertainty arising from sampling was 1.1%.

The contribution of uncertainty arising from sampling was (24%) lower than the uncertainty from analytical steps (76%). Prednisone is a BCS Class I drug (high solubility and high permeability), which may explain the reduced variability of the dissolved amount of API between units. According to ICH M19 guide, high variability in dissolution test is not expected for BCS Class I drugs (EMA, 2020b, 2020a). Uncertainty arising from sampling may be higher for drugs of BSC Classes II and IV

(low solubility) since it is expected a wider variability of the dissolved amount of API between units.

Since there is no equation model that explain the amount of prednisone dissolved as function of dissolution conditions (e.g. paddle rotation speed, volume of dissolution medium, time of dissolution, and pH of dissolution medium), this equation model was established experimentally using design of experiments (DoE). According to the analysis of variance (ANOVA) from DoE results (**Table 1**), the time of dissolution (X_3), the volume of dissolution medium (X_2), and the paddle rotation speed (X_1) affected the amount of prednisone dissolved. The pH of dissolution medium (X_4) did not affect the dissolution rate, which may be explain because the solubility of prednisone (pKa = 12.4) does not change with pH in the range from 4.0 to 9.6 (**Table 1**). In addition, there are significant interactions between paddle rotation speed and volume of dissolution medium (X_1X_2), between paddle rotation speed and time of dissolution (X_1X_3), and between volume of dissolution medium and time of dissolution (X_2X_3) (**Table 1**). The order of importance of each input factor (X_1, X_2, and X_3) and their interactions (X_1X_2, X_1X_3, and X_2X_3) into the output value (Y) is presented in Pareto chart (**Figure 3**).

Table 1. Analysis of variance (ANOVA) results for the dissolved amount of prednisone as a function of paddle rotation speed (X_1, from 40 to 60 rpm), volume of dissolution medium (X_2, from 800 to 1000 mL), time of dissolution (X_3, from 20 to 40 minutes), and pH of dissolution medium (X_4, from 4.0 to 9.6)

Source	d.f.	SS	MS	F-value	p-value
Regression	6	0.074341	0.012390	30.92	0.000
X_1	1	0.000138	0.000138	0.34	0.572
X_2	1	0.001199	0.001199	2.99	0.118
X_3	1	0.008999	0.008999	22.46	0.001
X_1X_2	1	0.002002	0.002002	4.99	0.052
X_1X_3	1	0.006251	0.006251	15.60	0.003
X_2X_3	1	0.001592	0.001592	3.97	0.077
Error	9	0.003607			
Total	15	0.077948			

Legend: d.f. = degrees of freedom, SS = sum of squares, and MS = mean squares

The regression model obtained was well adjusted, with significant regression (p-value < 0.001) and adjusted and prediction correlation coefficient values of 0.9229 and 0.8538, respectively. The measurement uncertainty from dissolution step was evaluated by Monte Carlo method simulation applied to the regression model previously obtained. Normally distributed random generators were used to simulate input values with mean and standard uncertainty, respectively, of 50 and 2 rpm for paddle rotation speed (X_1), of 900 and 10 mL for volume of dissolution medium (X_2), and of 30 and 2 minutes for time of dissolution (X_3). These values were defined in accordance with the requirements of general chapter <711> *Dissolution* of United States Pharmacopeia (USP) [11]. The standard uncertainty value for the dissolved amount of prednisone (output values) obtained from Monte Carlo methods was found to be 1.2%. Monte Carlo method was performed using 50,000 simulations. A schematic representation of Monte Carlo method applied to evaluate the uncertainty of dissolution step is presented in **Figure 4**.

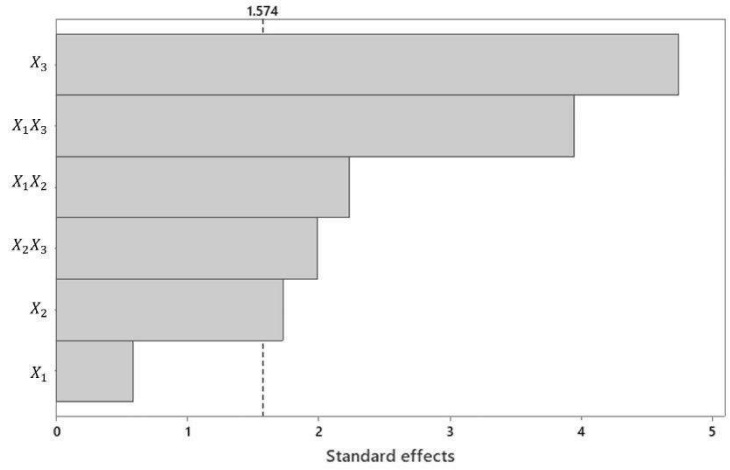

Figure 3. Pareto chart for the dissolved amount of prednisone as a function of paddle rotation speed (X_1, from 40 to 60 rpm), volume of dissolution medium (X_2, from 800 to 1000 mL), time of dissolution (X_3, from 20 to 40 minutes), and pH of dissolution medium (X_4, from 4.0 to 9.6).

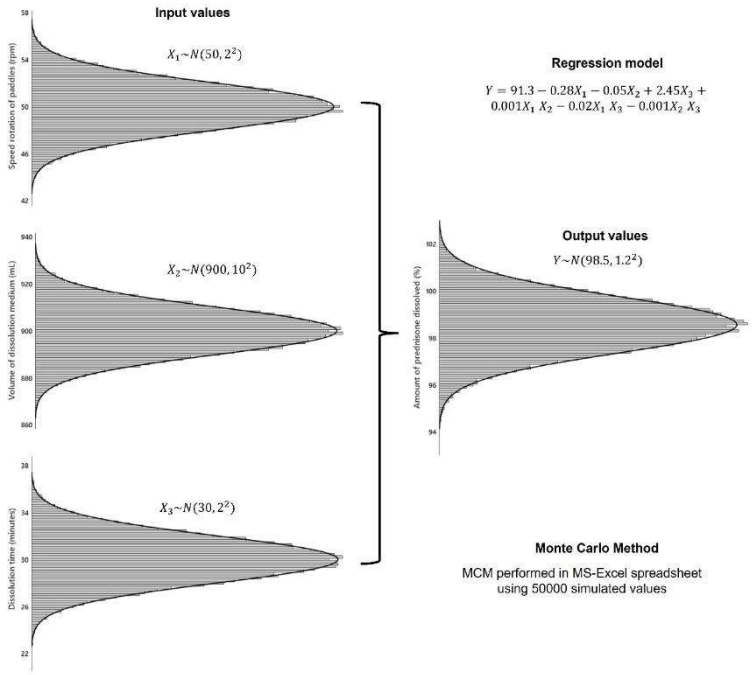

Figure 4. Schematic representation of Monte Carlo method applied to evaluate the uncertainty of dissolution step. Legend: X_1 = paddle rotation speed; X_2 = volume of dissolution medium; X_3 = time of dissolution; and Y = dissolved amount of prednisone.

The uncertainty associated with the dissolution step was mainly explained due to the time of dissolution, the paddle rotation speed, and volume of dissolution medium. Similar results were obtained for acetaminophen tablets (also BCS Class I drug), with paddle rotation speed and time of dissolution being the most relevant sources of uncertainty (ROMERO; LOURENÇO, 2017). The standard uncertainty value obtained for the dissolution of acetaminophen tablets was found to be 0.5%. It is important to note that only the uncertainties from dissolution step of acetaminophen tablets were considered (ROMERO; LOURENÇO, 2017).

Finally, the uncertainty arising from quantification step was calculated from linear least square calibration. The calibration curve was obtained using calibration standards with concentrations from 10 to 30 μg/mL. The standard uncertainty obtained for the quantification step was found to be 1.5% of the dissolved amount of prednisone.

The obtained uncertainty value (1.5%) is similar to the uncertainty value obtained for linezolid (1.1%); however, it is higher the uncertainty values obtained for desloratadine (0.6%), and acetamoniphen (0.6%) using UV spectrophotometry (FRANCISCO; SAVIANO; LOURENÇO, 2016; SAVIANO; LOURENÇO, 2013; TAKANO et al., 2017).

The overall standard uncertainty was calculated by combining the uncertainty values arising from sampling, dissolution and quantification steps. The contributions of uncertainty arising from sampling, dissolution, and quantification steps were found to be 24% ($u_s{}^2/u_{\%}{}^2$), 29% ($u_d{}^2/u_{\%}{}^2$) and 47% ($u_q{}^2/u_{\%}{}^2$), respectively (**Figure 5**). The combined standard uncertainty was found to be 2.2%, which is below the target uncertainty value (u^t = 2.5%) (BETTENCOURT DA SILVA; WILLIAMS, 2015).

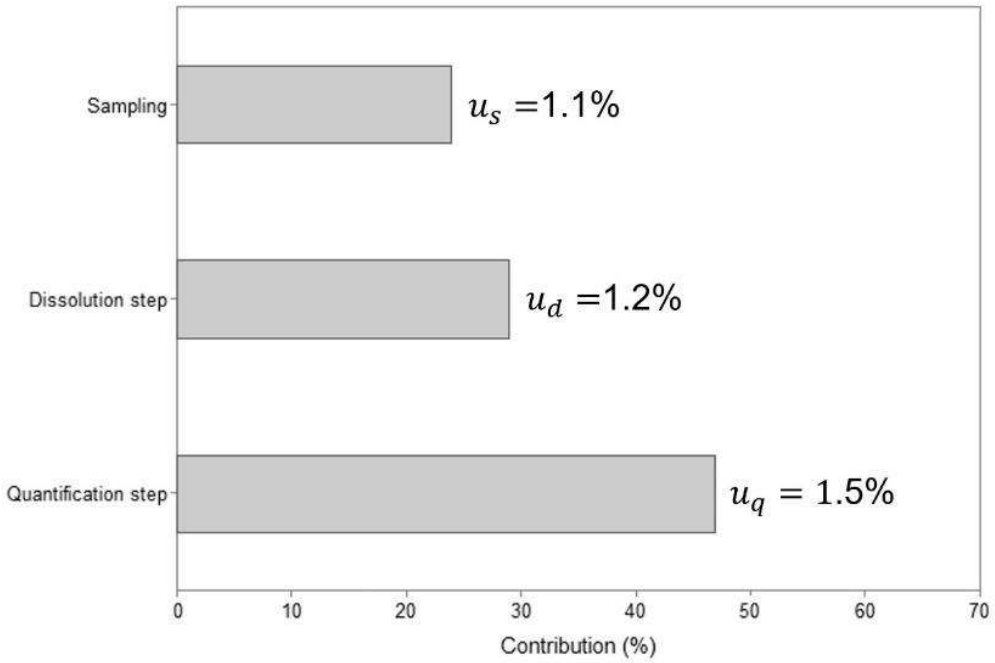

Figure 5. Pareto chart of the contributions of uncertainty arising from sampling (u_s), dissolution (u_d), and quantification (u_q) steps for the dissolution test of prednisone tablets.

4.3.2. Conformity assessment

The dissolved amount of prednisone obtained for 6 tablets of a commercial medicine batch is presented in **Table 2**.

Table 2. Measured values, specification limits, univariate and multivariate acceptance limits for the dissolution test of glyburide tablets.

# of tablets	Measured value and uncertainty	Specification limits	[1]Univariate acceptance limits	[2]Multivariate acceptance limits
1	96.5 ± 4.5%	85.0%	88.6%	90.2%
2	94.0 ± 4.5%	85.0%	88.6%	90.2%
3	96.4 ± 4.5%	85.0%	88.6%	90.2%
4	95.3 ± 4.5%	85.0%	88.6%	90.2%
5	96.0 ± 4.5%	85.0%	88.6%	90.2%
6	96.9 ± 4.5%	85.0%	88.6%	90.2%

Legend: [1]univariate acceptance limits = 85.0%+ku, where k=1.64 and u=2.2 and [2]multivariate acceptance limits = 85.0%+$k'u$, where k'=2.38 and u=2.2.

According to the requirements of stage 1 defined in general chapter <711> *Dissolution test* of United States Pharmacopeia (USP) (USP, 2021), the dissolved amount of active pharmaceutical ingredient (API, e.g. prednisone) for each of 6 units (e.g. tablets) must be not less the Q+5% (e.g. 85.0%, considering Q = 80.0%) (FARMACOPEIA BRASILEIRA, 2019; USP, 2021). Assuming a simple acceptance decision rule (also called shared risk decision rule), the batch complies with the specification of the dissolution test (**Figure 6**). However, when the measured values are close to the specification limits and/or if the uncertainty values are high, there may be a risk of false conformity decision (e.g. conclude that the batch complies with the specification limits when actually is out-of-specification).

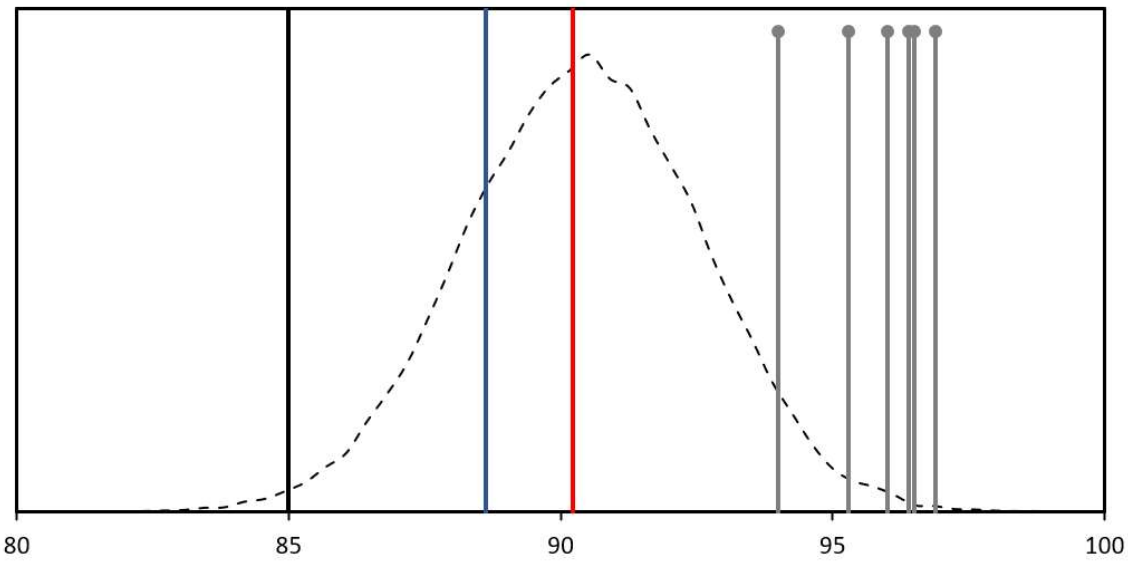

Figure 6. Measured values (|), specification limits (|), univariate (|) and multivariate (|) acceptance limits for the dissolution test of prednisone tablets.

Alternatively, a decision rule with use of guard-bands may be adopted to ensure a reduced risk of false decision. The guard-band width is calculated as the standard uncertainty multiplied by an appropriate coverage factor, and then the guard-band is summed up to the lower specification limit in order to obtain a restrict acceptance zone

that ensures a reduced consumer's risk (usually, up to 5%). Univariate and multivariate acceptance limits were found to be 88.6% and 90.2%, respectively (DA SILVA; LOURENÇO, 2023; DE OLIVEIRA; LOURENÇO, 2021). Univariate acceptance limit ensures a reduced particular risk (e.g. risk of false decision for a particular dosage unit), while multivariate acceptance limit ensures a reduced total risk (e.g. risk of false decision for the six dosage unit simultaneously). The measured values of all six tablets are within the univariate and multivariate acceptance limits, and thus the particular and total risk of false decisions are below 5% (**Figure 6**).

4.3. Conclusions

Measurement uncertainty of dissolution test for prednisone tablets was evaluated using a bottom-up approach. The main source of uncertainty was from the quantification step, followed by dissolution and sampling uncertainties; however, the contribution of these sources of uncertainty may be different for other active pharmaceutical ingredients (APIs). Combined uncertainty value was below target uncertainty value, which indicates the dissolution test provide proper results for conformity assessment. The measurement uncertainty information ensured a reduced risk of false conformity decisions.

uncertainty value, which indicates the dissolution test provide proper results for conformity assessment. The measurement uncertainty information ensured a reduced risk of false conformity decisions.

CAPÍTULO II

(CHAPTER II)

5. CHAPTER II - MEASUREMENT UNCERTAINTY ARISING FROM SAMPLING AND ANALYTICAL STEPS OF DISSOLUTION TEST OF GLYBURIDE TABLETS

ABSTRACT

The dissolution test is an essential technique used in pharmaceutical research to assess the rate and extent of drug release from a dosage form into a dissolution medium. Accurate and reliable dissolution test results are crucial to ensure the quality and efficacy of the drug product. The aim of this study was to evaluate the measurement uncertainty arising from the sampling and analytical steps of the dissolution test for glyburide tablets. Dissolution test was performed using 900 mL of phosphate buffer pH 7.3 as dissolution medium and a dissolution apparatus equipped with paddles rotating at 75 rpm for 60 minutes. Quantification was performed by HPLC-UV. The uncertainty arising from sampling was estimated through classical Analysis of Variance (ANOVA), using 8-sampling targets, two samples for each sampling target and two replicas for each sample, totalizing 32 analyses. Uncertainty arising from analytical steps considered the uncertainty from dissolution step (estimated using Monte Carlo method and regression equation obtained using DoE) and uncertainty from quantification step. The overall uncertainty value was found to be 6.33%. The sources of uncertainty contributing to the overall measurement uncertainty of the dissolution test were estimated to be 76.09% from sampling, 22.15% from the dissolution step, and 1.76% from the quantification step. The study emphasizes the importance of evaluating measurement uncertainty in dissolution testing to ensure reliable and accurate drug product quality control. The results demonstrate that the dissolution test of glyburide tablets presented high sampling uncertainty and did not pass the dissolution test within the specified limits, probably due to glyburide's low solubility. Univariate and multivariate rejection limits were calculated in order to ensure reduced particular and total producer's risk, respectively. This study provides useful insights for pharmaceutical industries to optimize their quality control processes and ensure the efficacy and safety of their products.

KEYWORDS: Dissolution test; measurement uncertainty; conformity assessment

5.1. Introduction

Dissolution tests of pharmaceutical forms plays a crucial role in drug development and quality control. It enables the quantification of the amount of drug released from the dosage form into a dissolution medium, which allows for the prediction of *in-vivo* drug performance and ensures batch-to-batch consistency. Moreover, the dissolution test serves as a bioequivalence indicator, enabling the identification of bioavailability issues and the determination of the necessity for bioequivalence testing (SHAH, 2001; STORPIRTIS et al., 2004)

Introduced in the 1900s, the Biopharmaceutical Classification System (BCS) developed by Amidon and colleagues is based on fundamental properties governing drug absorption. BCS is a classification system that groups drugs based on their solubility and permeability characteristics to predict oral bioavailability, a measure of drug absorption into the bloodstream following oral administration. The system categorizes drugs into four classes: Class I (high solubility, high permeability), Class II (low solubility, high permeability), Class III (high solubility, low permeability), and Class IV (low solubility, low permeability) (AMIDON et al., 1995; DOKOUMETZIDIS; MACHERAS, 2006).

For class II drugs, which are expected to have a slower dissolution rate, the dissolution test is particularly important, since the dissolution of these drugs is the limiting factor for their absorption, consequently affecting their bioavailability (COOK; ADDICKS; WU, 2008; DOKOUMETZIDIS; MACHERAS, 2006).

The dissolved amount of active pharmaceutical ingredient (API) should be compared to a set of acceptance criteria, such as pharmacopeial standards or in-house specifications, to ensure that the medicine meets the desired quality standards. The

dissolution test should be performed on a representative sample of the drug product to ensure that the results are representative of the entire batch (EUROPEAN PHARMACOPEIA (PH. EUR.), 2020; FARMACOPEIA BRASILEIRA, 2019; USP, 2021).

Given the significance of dissolution test results in assessing the conformity of a medication, it is crucial to evaluate the quality of these results. It is increasingly recognized and necessary to demonstrate the quality of measurement, as inadequate analysis data can lead to disastrous consequences (ROMERO; LOURENÇO, 2017; SEPAROVIC et al., 2023).

Measurement uncertainty is a crucial parameter that reflects the degree of confidence or probability that the true value of a measured quantity falls within a certain range (BETTENCOURT DA SILVA; WILLIAMS, 2015; ELLISON; WILLIAMS, 2012; RAMPSEY; ELLISON; ROSTRON, 2019). In pharmaceutical analysis, managing and reducing measurement uncertainty is of utmost importance, as it can significantly affect the interpretation of results and the decisions based on those results (ELLISON; WILLIAMS, 2012; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2015, 2017; RAMPSEY; ELLISON; ROSTRON, 2019). Measurement uncertainty in pharmaceutical analysis can arise from both the sampling and analytical steps of the measurement process (ELLISON; WILLIAMS, 2012; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2015, 2017; SEPAROVIC et al., 2023; TRAPLE et al., 2014). Uncertainty arising from sampling may be due to several factors such as the heterogeneity of the sample, the sampling method used, and the sample

preparation process. On the other hand, uncertainty arising from analytical step may be due to instrument calibration, measurement errors, random variations, and environmental conditions (ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2015, 2017, 2023).

The combined uncertainty can be obtained using either the bottom-up or top-down approaches. The bottom-up approach combines the systematic and random errors of each step of the analytical procedure to obtain the overall measurement uncertainty, while the top-down approach combines the systematic and random errors of the analytical procedure as a whole to obtain the measurement uncertainty (SEPAROVIC et al., 2023). By using measurement uncertainty information, it is possible to evaluate different types of risks such as consumer's and producer's risks, global and specific risks, particular and total risks (BETTENCOURT DA SILVA et al., 2019; COMMITTEE FOR GUIDES IN METROLOGY, 2012; DA SILVA; LOURENÇO, 2023; FRANCISCO; SAVIANO; LOURENÇO, 2016; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2023; SAVIANO; LOURENÇO, 2018). Consumer's risk is the risk of incorrectly accepting a poor-quality lot (with a result outside the specification limits), while producer's risk is the risk of rejecting a good quality lot (with a result within the specification limits).

Guard-bands can be adopted to minimize the risk of false conformity decisions in pharmaceutical analysis (COMMITTEE FOR GUIDES IN METROLOGY, 2012; DA SILVA; LOURENÇO, 2023). Guard-bands are an additional margin of safety or assurance added or subtracted to the specification limits, which reduces the probability of false conformity decisions. This is particularly important when there is a risk of harm

to human health or safety if a product fails to meet the required standards. Guard-bands are typically established based on the level of risk involved, adopting an appropriate coverage factor, and the available knowledge about the measurement results (BETTENCOURT DA SILVA; WILLIAMS, 2015; DA SILVA; LOURENÇO, 2023; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2023).

The objective of this study is to evaluate the measurement uncertainty arising from the sampling and analytical steps of the dissolution test of glyburide tablets. Furthermore, measurement uncertainty is used to establish guard-bands to ensure an increased probability of correct acceptance, thereby reducing producer's risk.

5.2. Materials and Methods

5.2.1. Dissolution test of glyburide tablets

Glyburide tablet samples were purchased in Brazilian marked. Glyburide certified reference substance (CRS) was obtained from United States Pharmacopoeia (USP) (USP, 2021).

Dissolution test was performed using 900 mL of phosphate, pH 7.3 as dilution medium, using a dissolution apparatus (Nova Ética, 299) equipped with paddles rotating at 75 rpm. After 60 minutes, 5-10 mL aliquots of dissolution medium were withdrawn and properly diluted using purified water as diluent. Glyburide quantification was performed using a HPLC-UV (Thermo, Acella) utilizing a column C-8 Zorbax, 250 mm

wide x 4.6 mm of internal diameter, and 5 µm of particule size. The mobile phase was mixture of acetonitrile and purified water (1:1), containing 4.0 mL of phosphoric acid per liter, at a flow rate 1.5mL/min. The volume of injection of the sample was 50 µL. The amount of glyburide dissolved for each tablet was calculated by comparing the area of the peaks obtained from of sample solutions to the area of the peaks obtained from glyburide CRS solutions at concentrations from 2 to 6 µg/mL.

According to the pharmacopeial requirements, the amount of glyburide dissolved for each of six tablets must be not less than 75% (Q = 70%, in stage 1 all dissolved amount for all tablets must be not less than Q+5%) of the label content (EUROPEAN PHARMACOPEIA (PH. EUR.), 2020; FARMACOPEIA BRASILEIRA, 2019; USP, 2021).

5.2.2. Uncertainty arising from sampling

Uncertainty arising from sampling was estimated using the duplicate method (empirical approach), which is a simple and most cost-effective method. In the duplicate method, 8-sampling target were subject to dissolution test. For each of the 8-sampling target, two samples were collected, resulting in 16 samples. All 16 samples were analyzed two times (duplicates), resulting in 32 analyses. The random component of the uncertainty arising from sampling was estimated by classical Analysis of Variance (ANOVA) utilizing the spreadsheet available in the Eurachem/CITAC guide on measurement uncertainty arising from sampling (RAMPSEY; ELLISON; ROSTRON, 2019).

5.2.3. Uncertainty arising from analytical steps

Uncertainty arising from analytical steps were divided in two main sources of uncertainty: 1) uncertainty from dissolution step; and 2) uncertainty from quantification step. Both steps were studied in detail as described below.

The measurement uncertainty from dissolution step was performed Monte Carlo method in association with a regression model that explains the amount of glyburide dissolved as a function of pH (A, From 6.30 to 8.05), paddle rotation speed (B, from 50 to 100 rpm), time of dissolution (C, from 50 to 70 minutes) and volume of dissolution medium (D, from 800 to 1000 mL). The regression model was obtained from experimental data, using a factorial design (FRANCISCO; SAVIANO; LOURENÇO, 2016; ROMERO; LOURENÇO, 2017; SAVIANO; LOURENÇO, 2018)

The evaluation of uncertainties arising from the quantification step considered the uncertainty from linear least squares calibration (ELLISON; WILLIAMS, 2012), using **Eq. (3)**:

$$u_q = \frac{s}{b}\sqrt{\frac{1}{p}+\frac{1}{n}+\frac{(q-\bar{c})^2}{\sum_i^n (c_i-\bar{c})^2}} \qquad \text{Eq. (3)}$$

Where, q and u_{q} is the concentration of glyburide in the sample solution and its respective uncertainty, s is the residual standard deviation, b is the slope of least square regression, p and n are the number of measurements for sample and standard solutions, c_i is the concentration of the calibration standard corresponding to the i-th measurement (n number of measurements), and $\bar{c}$ is the mean value of the calibration standards (n number of measurements).

5.2.3. Combined measurement uncertainty

The combined measurement uncertainty was obtained using the law of uncertainty propagation (ELLISON; WILLIAMS, 2012; SEPAROVIC et al., 2023), using **Eq. (4)**.

$$u_{\%} = \sqrt{{u_s}^2 + {u_d}^2 + {u_q}^2} \qquad \text{Eq. (4)}$$

Where, $u_{\%}$ is the combined uncertainty of the dissolution test for glyburide tablets, u_s is the uncertainty arising from sampling, u_d is the uncertainty from dissolution step, and u_q is the uncertainty from the quantification step.

The expanded uncertainty ($U_{\%}$) was obtained by multiplying the combined uncertainty ($u_{\%}$) by an appropriate coverage factor (k). Typically, k = 2 is used for an approximately 95% confidence level.

5.2.4. Conformity assessment

Univariate (g) and multivariate (g') guard-band widths were calculated by multiplying the combined measurement uncertainty by appropriate coverage factors (k and k' for univariate and multivariate guard bands, respectively). Coverage factor values used were 2.33 and 0.08 for univariate and multivariate guard bands, respectively, for an approximately 99% confidence level (BETTENCOURT DA SILVA; LOURENÇO;

HIBBERT, 2022; DA SILVA; LOURENÇO, 2023; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2023; WILLIAMS et al., 2021).

The guard-band widths (g and g') were subtracted to the specification limit (Q+5% = 75% for stage 1) to obtained univariate and multivariate rejection limits (R = 75%$-g$ and R' = 75%$-g'$). The product should be declared non-compliant if the dissolved amount of glyburide for all six tablets are below the multivariate rejection limits. In this case, the total producer's risk of false decisions should be less than 1%.

5.3. Results and Discussion

5.3.1. Uncertainty arising from sampling and analytical steps

The uncertainty arising from sampling was evaluated by collecting two samples from each of 8 sampling targets and analyzing them in duplicate, resulting in 32 analyses. Analysis of variance (ANOVA) results indicated that the uncertainty arising from sampling was 5.4%, which was higher than the uncertainty from analytical steps. This may be due to the fact that glyburide is a BCS Class II drug with low solubility and high permeability. Thus, a high variability in dissolved amount between samples was expected. However, drugs of BSC Classes I and III (high solubility) may exhibit lower dissolution variability.

To establish a model for predicting the amount of glyburide dissolved as a function of dissolution conditions, a design of experiments (DoE) approach was

employed. ANOVA results (**Table 3**) showed that paddle rotation speed, volume of dissolution medium, time of dissolution and the correlation of paddle rotation speed/volume of dissolution medium have significantly affected the amount of glyburide dissolved **(Figure 7)**. Monte Carlo simulation applied to the regression model obtained from the DoE approach revealed that the standard uncertainty value for the dissolved amount of glyburide was 2.98%, mainly due to the uncertainty associated with the dissolution medium pH, time of dissolution, paddle rotation speed, and volume of dissolution medium. The significant level of uncertainty associated with the dissolution step can be attributed to various factors, such as the solubility of glyburide in the dissolution medium and the pH of the buffer solution used in the experiment. Due to glyburide's weak acid properties, it displays higher solubility in basic aqueous environments. In the DoE, the buffer solution employed had a pH ranging from 6.3 to 8.05, which can affect the solubility of glyburide given its pKa of 5.3 (WEI; LÖBENBERG, 2006) and, consequently, the dissolution rate of the tablets. The intricate nature of the dissolution process, which can be influenced by several factors, including agitation rate, temperature, and sample collection method, can also contribute to a significant degree of uncertainty in the obtained results. Therefore, the combined effects of the buffer solution pH and the complex dissolution process can lead to a considerable level of uncertainty in the glyburide tablets dissolution test.

Table 3. Analysis of variance (ANOVA) results for the dissolved amount of glyburide as a function of pH (A, From 6.30 to 8.05), paddle rotation speed (B, from 50 to 100 rpm), time of dissolution (C, from 50 to 70 minutes) and volume of dissolution medium (D, from 800 to 1000 mL).

Source	d.f.	SS	MS (Aj.)	F-value	p-value
Regression	6	6.44406	1.07401	249.94	0
C	1	0.01531	0.01531	3.56	0.07
A	1	0.04372	0.04372	10.18	0.004
B	1	0.01591	0.01591	3.7	0.065
D	1	0.15046	0.15046	35.01	0
AA	1	0.0899	0.0899	20.92	0
AD	1	0.20528	0.20528	47.77	0
Error	27	0.11602	0.0043		
Total	33	6.56008			

Legend: d.f. = degrees of freedom, SS = sum of squares, and MS = mean squares

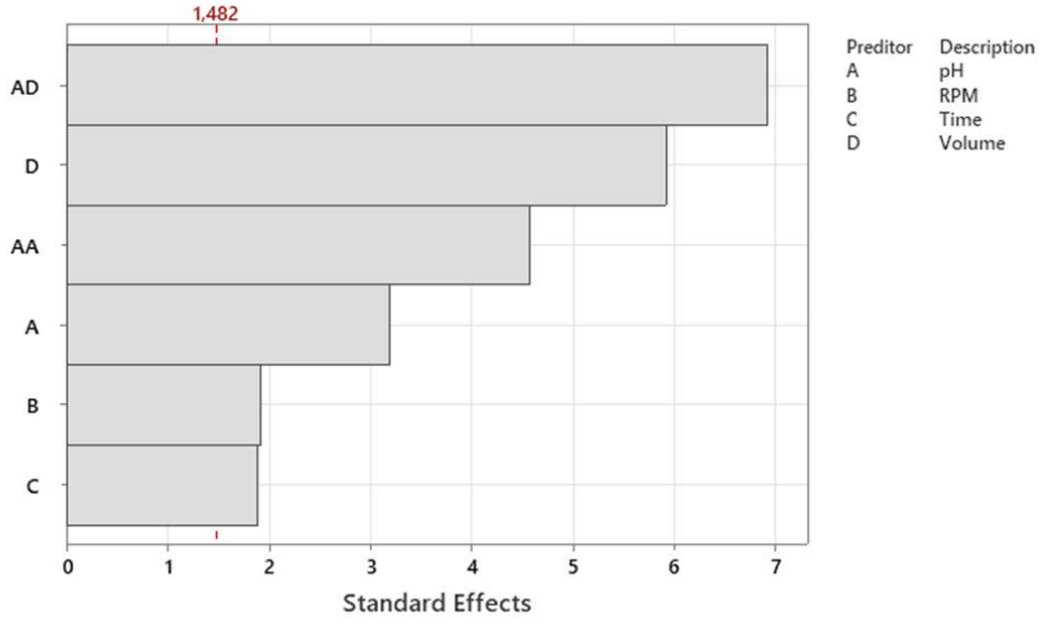

Figure 7. Pareto chart for the dissolved amount of glyburide as a function of pH (A, From 6.30 to 8.05), paddle rotation speed (B, from 50 to 100 rpm), time of dissolution (C, from 50 to 70 minutes) and volume of dissolution medium (D, from 800 to 1000 mL).

The uncertainty associated with the quantification step was evaluated using linear least square calibration. The obtained standard uncertainty value for the quantification step was 0.84% of the dissolved amount of glyburide. The overall standard uncertainty was calculated by combining the uncertainty values arising from sampling, dissolution, and quantification steps. The contributions of uncertainty arising from sampling, dissolution, and quantification steps were found to be 76.09%, 22.15%, and 1.76% (**Figure 8**), respectively, with a combined standard uncertainty of 6.3%, which was above the target uncertainty value.

Considering that the uncertainty arising from sampling and dissolution step are the most relevant sources of uncertainty, the overall uncertainty evaluation may be simplified and takes into account only the uncertainty from sampling and dissolution step. However, this simplification may not be proper for other active pharmaceutical ingredients, particularly for BCS I and III.

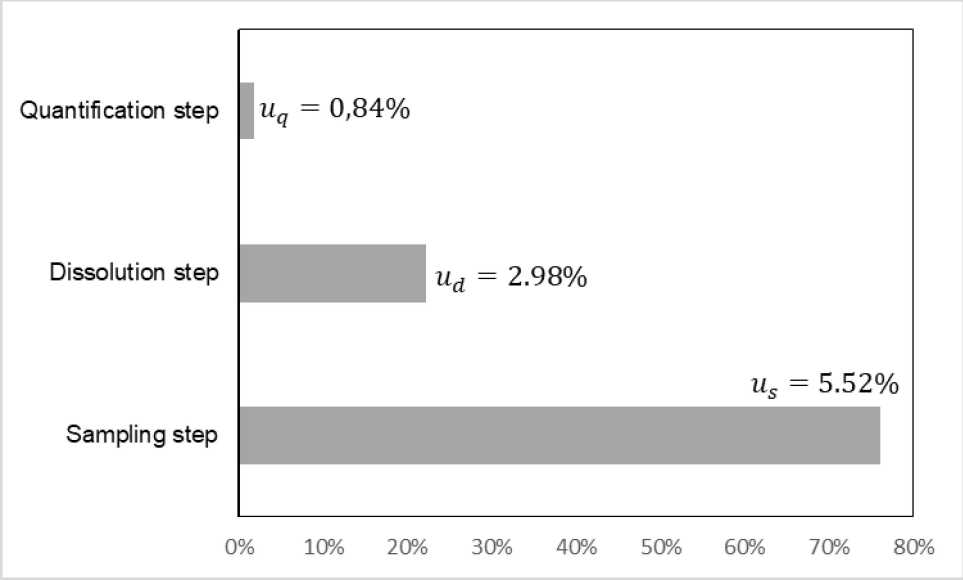

Figure 8. Pareto chart of the contributions of uncertainty arising from sampling (u_s), dissolution (u_d), and quantification (u_q) steps for the dissolution test of glyburide tablets.

5.3.2. Conformity assessment

The dissolved amount of glyburide was determined for six tablets of a commercial medicine batch, and the results are presented in **Table 4**. The requirements of stage 1, as outlined in the general chapter <711> Dissolution test of the United States Pharmacopeia (USP) (USP, 2021), dictate that the dissolved amount of the active pharmaceutical ingredient (API), glyburide in this case, must not be less than Q+5% for each of the six units (tablets). Assuming a simple decision rule (also called shared risk decision rule), the batch does not comply with the specification of the dissolution test (**Figure 9**). However, it is important to note that when measured values are in close proximity to the specification limits and/or the uncertainty values are high, there may be a risk of a false decision, where the batch is deemed non-compliant when it is actually within specification (producer's risk).

Table 4. Measured values, specification limits, univariate and multivariate rejection limits for the dissolution test of glyburide tablets.

# of tablets	Measured value and uncertainty	Specification limits	[1]Univariate rejection limits	[2]Multivariate rejection limits
1	60.6 ± 6.3%	75.0%	60.3%	74.3%
2	60.5 ± 6.3%	75.0%	60.3%	74.3%
3	60.0 ± 6.3%	75.0%	60.3%	74.3%
4	57.8 ± 6.3%	75.0%	60.3%	74.3%
5	53.2 ± 6.3%	75.0%	60.3%	74.3%
6	56.9 ± 6.3%	75.0%	60.3%	74.6%

Legend: [1]univariate rejection limits = $75.0\% - ku$, where k=2.33 and u=6.3 and [2]multivariate rejection limits = $75.0\% - k'u$, where k'=0.11 and u=6.3.

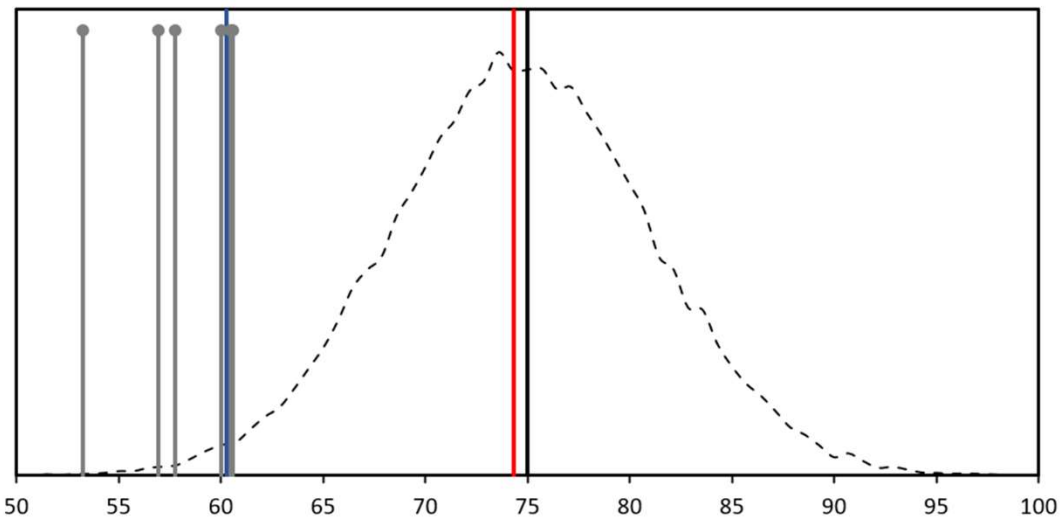

Figure 9. Measured values (|), specification limits (┃), univariate (|) and multivariate (|) acceptance limits for the dissolution test of glyburide tablets.

To minimize the risk of false decisions, a decision rule incorporating guard-bands can be adopted. The guard-band width is determined by multiplying the standard uncertainty by an appropriate coverage factor and then adding this value to the lower specification limit. This ensures a restricted acceptance zone that minimizes the producer's risk, typically up to 5%. Univariate and multivariate rejection limits were found to be 60.3% and 74.3%, respectively (BETTENCOURT DA SILVA et al., 2019; DA SILVA; LOURENÇO, 2023; ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE), 2023). The univariate rejection limit ensures a reduced particular risk (i.e., the risk of a false decision for a particular dosage unit), while the multivariate rejection limit ensures a reduced total risk (i.e., the risk of a false decision for all six dosage units simultaneously). Notably, the measured values of four glyburide tablets were found to be out of the univariate rejection limit, however the measured values of two glyburide tablets were found to be between the univariate and multivariate rejection limits. All six measured values of glyburide tablets were below

the multivariate rejection limit, indicating that total risk of false decision was below 1% (**Figure 9**).

5.4. Conclusions

Measurement uncertainty evaluation of dissolution test for glyburide tablets was performed using a bottom-up approach. The uncertainty arising from sampling and dissolution step were the most relevant sources of uncertainty, contributing to almost 76% and 22% respectively of overall uncertainty. Thus, the overall measurement uncertainty evaluation may be simplified and consider only the uncertainty arising from sampling and dissolution step as the contribution of quantification step is negligible. However, this simplification may not be proper for other active pharmaceutical ingredients (API). The results of this study can be useful for improving the accuracy and reliability of dissolution test results, which can ultimately lead to better drug development and quality control.

CONCLUSÃO

6. CONCLUSÃO

A avaliação da incerteza de medição no teste de dissolução para comprimidos de diferentes ingredientes farmacêuticos ativos (IFAs) foi realizada utilizando uma abordagem de *bottom-up*. Embora os resultados tenham variado para os diferentes APIs estudados, ambos os estudos destacaram a importância dessa avaliação para melhorar a confiabilidade dos resultados do teste de dissolução.

No estudo da incerteza do teste de dissolução de comprimidos de prednisona, a principal fonte de incerteza foi a etapa de quantificação, seguida pelas incertezas relacionadas às etapas de dissolução e de amostragem. A contribuição dessas fontes de incerteza pode variar para diferentes IFAs. Além disso, observou-se que o valor combinado de incerteza estava abaixo do valor de incerteza-alvo, indicando que o teste de dissolução fornece resultados adequados para a avaliação de conformidade. A informação sobre a incerteza de medição assegurou uma redução no risco de decisões falsas de conformidade.

Já no estudo com comprimidos de glibenclamida, as fontes mais relevantes de incerteza foram as etapas de amostragem e dissolução, representando cerca de 76% e 22% da incerteza geral, respectivamente. Desta forma, a avaliação da incerteza de medição poderia ser simplificada, considerando apenas essas duas etapas, devido à insignificante contribuição da etapa de quantificação. No entanto, essa simplificação pode não ser adequada para outros IFAs.

Em conclusão, os estudos enfatizaram a importância da avaliação da incerteza de medição no teste de dissolução, destacando diferentes fontes de incerteza e suas contribuições específicas para cada IFA estudado. Uma avaliação adequada da incerteza

de medição pode proporcionar resultados mais confiáveis dos testes de dissolução, diminuindo os riscos do produtor e do consumidor quanto a tomada de decisões falsas.

7. REFERÊNCIAS BIBLIOGRÁFICAS

AMIDON, Gordon L.; LENNERNÄS, Hans; SHAH, Vinod P.; CRISON, John R. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. **Pharmaceutical Research**, *[S. l.]*, v. 12, n. 3, p. 413–420, 1995. DOI: 10.1023/A:1016212804288.

BETTENCOURT DA SILVA, R. J. N.; WILLIAMS, A. **Eurachem/CITAC Guide: Setting and Using Target Uncertainty in Chemical Measurement Eurachem/CITAC Guide Setting and Using Target Uncertainty in Chemical Measurement First edition**. [s.l: s.n.]. Disponível em: www.eurachem.org.

BETTENCOURT DA SILVA, Ricardo J. N.; LOURENÇO, Felipe; HIBBERT, D. Brynn. Setting Multivariate and Correlated Acceptance Limits for Assessing the Conformity of Items. **Analytical Letters**, *[S. l.]*, v. 55, n. 13, p. 2011–2032, 2022. DOI: 10.1080/00032719.2022.2042549.

BETTENCOURT DA SILVA, Ricardo J. N.; LOURENÇO, Felipe R.; PENNECCHI, Francesca R.; HIBBERT, D. Brynn; KUSELMAN, Ilya. Spreadsheet for evaluation of global risks in conformity assessment of a multicomponent material or object. **Chemometrics and Intelligent Laboratory Systems**, *[S. l.]*, v. 188, p. 1–5, 2019. DOI: 10.1016/j.chemolab.2019.02.010.

COMMITTEE FOR GUIDES IN METROLOGY, Joint. **Evaluation of measurement data-The role of measurement uncertainty in conformity assessment Évaluation des données de mesure-Le rôle de l'incertitude de mesure dans l'évaluation de la conformité**. [s.l: s.n.]. Disponível em: https://www.bipm.org/. Acesso em: 30 mar. 2023.

COOK, Jack; ADDICKS, William; WU, Yunhui Henry. Application of the Biopharmaceutical Classification System in Clinical Drug Development—An Industrial View. **The AAPS Journal**, *[S. l.]*, v. 10, n. 2, p. 306–310, 2008. DOI: 10.1208/s12248-008-9036-5.

DA SILVA, Cintia Margueiro; LOURENÇO, Felipe Rebello. Definition of multivariate acceptance limits (guard-bands) applied to pharmaceutical equivalence assessment. **Journal of Pharmaceutical and Biomedical Analysis**, *[S. l.]*, v. 222, p. 115080, 2023. DOI: 10.1016/j.jpba.2022.115080.

DE OLIVEIRA, Elcio Cruz; LOURENÇO, Felipe Rebello. Risk of false conformity assessment applied to automotive fuel analysis: A multiparameter approach. **Chemosphere**, *[S. l.]*, v. 263, p. 128265, 2021. DOI: 10.1016/j.chemosphere.2020.128265.

DOKOUMETZIDIS, Aristides; MACHERAS, Panos. A century of dissolution research: From Noyes and Whitney to the Biopharmaceutics Classification System.

International Journal of Pharmaceutics, *[S. l.]*, v. 321, n. 1–2, p. 1–11, 2006. DOI: 10.1016/j.ijpharm.2006.07.011.

ELLISON, S. R. L.; WILLIAMS, A. **Eurachem/CITAC Guide: Quantifying uncertainty in analytical measurement**. 3. ed. [s.l.] : www.eurachem.org, 2012. Disponível em: www.eurachem.org. Acesso em: 30 mar. 2023.

EMA. **Committee for Medicinal Products for Human Use ICH M9 guideline on biopharmaceutics classification system-based biowaivers-questions and answers**. [s.l: s.n.]. Disponível em: www.ema.europa.eu/contact.

EMA. **Committee for Medicinal Products for Human Use ICH M9 guideline on biopharmaceutics classification system-based biowaivers ICH M9 on biopharmaceutics classification system-based biowaivers**. [s.l: s.n.]. Disponível em: www.ema.europa.eu/contact.

EUROPEAN PHARMACOPEIA (PH. EUR.). European Directorate for the Quality of Medicines & HealthCare. *Em*: , 2020.

FARMACOPEIA BRASILEIRA. Agência Nacional de Vigilância Sanitária (ANVISA). *Em*: , 2019.

FDA. **Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System Guidance for Industry**. [s.l: s.n.]. Disponível em: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

FRANCISCO, Fabiane Lacerda; SAVIANO, Alessandro Morais; LOURENÇO, Felipe Rebello. Measuring uncertainty using different approaches: A case study for acetaminophen quantification. **Journal of AOAC International**, *[S. l.]*, v. 99, n. 3, p. 612–617, 2016. DOI: 10.5740/jaoacint.15-0199.

ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE). **ICH guideline Q10 on pharmaceutical quality system. Q10**. 2015.

ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE). **ICH guideline Q8 (R2) on pharmaceutical development. Q8(R2)**. 2017.

ICH (INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE). **ICH guideline Q9 (R1) on quality risk management. Q9(R1)**. 2023.

JCGM 106:2012. **Evaluation of measurement data - The role of measurement uncertainty in conformity assessment**. [s.l: s.n.]. Disponível em: https://www.gov.br/inmetro/pt-br/centrais-de-.

KUSELMAN, Ilya; PENNECCHI, Francesca; DA SILVA, Ricardo J. N. B.; BRYNN HIBBERT, D. Conformity assessment of multicomponent materials or objects: Risk of false decisions due to measurement uncertainty – A case study of denatured alcohols. **Talanta**, *[S. l.]*, v. 164, p. 189–195, 2017. a. DOI: 10.1016/j.talanta.2016.11.035.

KUSELMAN, Ilya; PENNECCHI, Francesca R.; DA SILVA, Ricardo J. N. B.; HIBBERT, D. Brynn. Risk of false decision on conformity of a multicomponent material when test results of the components' content are correlated. **Talanta**, *[S. l.]*, v. 174, p. 789–796, 2017. b. DOI: 10.1016/j.talanta.2017.06.073.

PENNECCHI, Francesca R.; KUSELMAN, Ilya; DA SILVA, Ricardo J. N. B.; HIBBERT, D. Brynn. Risk of a false decision on conformity of an environmental compartment due to measurement uncertainty of concentrations of two or more pollutants. **Chemosphere**, *[S. l.]*, v. 202, p. 165–176, 2018. DOI: 10.1016/j.chemosphere.2018.03.054.

RAMPSEY, M. H.; ELLISON, S. L. R.; ROSTRON, P. **Eurachem/CITAC Guide: Measurement uncertainty arising from sampling**. 2. ed. [s.l.] : www.eurachem.org, 2019. Disponível em: www.eurachem.org. Acesso em: 30 mar. 2023.

ROMERO, Daniel Cancelli; LOURENÇO, Felipe Rebello. Measurement uncertainty of dissolution test of acetaminophen immediate release tablets using Monte Carlo simulations. **Brazilian Journal of Pharmaceutical Sciences**, *[S. l.]*, v. 53, n. 3, 2017. DOI: 10.1590/s2175-97902017000300163.

SAVIANO, Alessandro Morais; LOURENÇO, Felipe Rebello. Uncertainty evaluation for determining linezolid in injectable solution by UV spectrophotometry. **Measurement**, *[S. l.]*, v. 46, n. 10, p. 3924–3928, 2013. DOI: 10.1016/j.measurement.2013.08.005.

SAVIANO, Alessandro Morais; LOURENÇO, Felipe Rebello. Measurement uncertainty estimation based on multiple regression analysis (MRA) and Monte Carlo (MC) simulations – Application to agar diffusion method. **Measurement**, *[S. l.]*, v. 115, p. 269–278, 2018. DOI: 10.1016/j.measurement.2017.10.057.

SEPAROVIC, Luciana; SIMABUKURO, Renan S.; COUTO, Aldo R.; BERTANHA, Maria Luiza G.; DIAS, Francielle R. S.; SANO, Adriano Y.; CAFFARO, Arthur M.; LOURENÇO, Felipe R. Measurement Uncertainty and Conformity Assessment Applied to Drug and Medicine Analyses – A Review. **Critical Reviews in Analytical Chemistry**, *[S. l.]*, v. 53, n. 1, p. 123–138, 2023. DOI: 10.1080/10408347.2021.1940086.

SHAH, Vinod P. Dissolution: A Quality Control Test vs. A Bioequivalence Test. **Dissolution Technologies**, *[S. l.]*, v. 8, n. 4, p. 6–7, 2001. DOI: 10.14227/DT080401P6.

STORPIRTIS, Sílvia; MARCOLONGO, Raquel; GASPAROTTO, Fernanda S.; VILANOVA, Crisálida M. **A equivalência farmacêutica no contexto da intercambialidade entre medicamentos genéricos e de referência: bases técnicas e científicas& Balant**. [s.l.] : Meyer, 2004.

TAKANO, Diogo Eiji Nakao; DE SOUZA REIS, Pedro Rodrigo; SINGH, Anil Kumar; LOURENÇO, Felipe Rebello. Estimation of uncertainty for measuring desloratadine in tablets formulation using UV spectrophotometry. **Measurement**, *[S. l.]*, v. 101, p. 40–44, 2017. DOI: 10.1016/j.measurement.2017.01.018.

TRAPLE, Marcus Augusto Lyrio; SAVIANO, Alessandro Morais; FRANCISCO, Fabiane Lacerda; LOURENÇO, Felipe Rebello. Measurement uncertainty in pharmaceutical analysis and its application. **Journal of Pharmaceutical Analysis**, *[S. l.]*, v. 4, n. 1, p. 1–5, 2014. DOI: 10.1016/j.jpha.2013.11.001.

USP. United States Pharmacopeial Convention. *Em*: , 2021.

WEI, Hai; LÖBENBERG, Raimar. Biorelevant dissolution media as a predictive tool for glyburide a class II drug. **European Journal of Pharmaceutical Sciences**, *[S. l.]*, v. 29, n. 1, p. 45–52, 2006. DOI: 10.1016/j.ejps.2006.05.004.

WILLIAMS, Alex et al. **Use of Uncertainty Information in Compliance Assessment Second edition (2021) Editors Composition of the Working Group* Eurachem members A. Brzyski CITAC members**. [s.l: s.n.]. Disponível em: www.eurachem.org.

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