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Article

Multivariate guard-bands and total risk assessment on multiparameter evaluations with correlated and uncorrelated measured values

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The quality, efficacy, and safety of medicines are usually verified by analytical results. Measurement uncertainty is a critical aspect for the reliability of these analytical results. The pharmacopeial compendia usually adopt a simple acceptance rule that does not consider information from measurement uncertainty. In this work, we compared decision-making using simple acceptance and decision rules with the use of guard-band for multiparameter evaluation of ofloxacin ophthalmic solution and acyclovir topical cream. Ciprofloxacin ophthalmic solution and acyclovir topical cream samples were subject to pharmacopeial tests and assays. Multivariate guard-band widths were calculated by multiplying the standard uncertainty (u) by an appropriate multivariate coverage factor (k'). The multivariate coverage factor (k') was obtained by the Monte Carlo method. According to the simple acceptance rule, all the results obtained for ciprofloxacin ophthalmic solution and acyclovir topical cream are within the specification limits. However, the risk of false conformity decisions increases for ciprofloxacin tests. Decisions made using the simple acceptance rule and decision rules with the use of guard-band may differ. The simple acceptance rule may increase the risk of false conformity decisions when the measured value is close to the regulatory specification limits and/or when the measurement uncertainty value is inappropriately high. Nevertheless, the guard-band decision rule will always reduce the risk of false conformity decisions. Therefore, using information on measurement uncertainty in conformity assessment is highly recommended to ensure the proper efficacy, safety, and quality of medicines.

Keywords: Measurement uncertainty. Conformity assessment. Multivariate analysis.

INTRODUCTION

Medicines are essential for maintaining good health and treating diseases and illnesses. The quality, efficacy, and safety of medicines are critical factors that determine their effectiveness to provide relief and cure. Quality refers to the level of excellence or superiority of a product, and in the case of medicines, it encompasses the identity, purity, strength, and composition of a drug (ICH Q8(R2), 2017; ICH Q9(R1), 2023; ICH Q10, 2015). Quality medicines are critical to ensure that the patient receives the intended dose of the active pharmaceutical ingredient (API), which in turn ensures the desired therapeutic effect. Quality control is a crucial step in manufacturing medicines and considers a series of analytical results to ensure that the medicine meets the established standards (Bertanha, Lourenço, 2021; Lombardo, da Silva, Lourenço, 2022).

To ensure that the quality, efficacy, and safety of medicines are maintained, they are subject to strict regulations and monitoring. In most countries, the regulatory authority responsible for overseeing the pharmaceutical industry is agencies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Regulatory agencies are responsible for ensuring that drugs are safe, effective, and of high quality, and they set standards for drug manufacturing, labeling, and

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marketing (United States Pharmacopeia, 2021; European Pharmacopeia, 2020; Farmacopeia Brasileira, 2019).

In addition to regulatory oversight, the pharmaceutical industry is also responsible for ensuring the quality, efficacy, and safety of its products. This includes maintaining rigorous quality control procedures in their manufacturing processes, as well as analytical development and validation (ICH Q2(R2), 2022; ICH Q14, 2022).

Measurement uncertainty is a critical aspect of the quality, efficacy, and safety of medicines, as it can be used to assess the risk of false conformity decisions that could have serious consequences (Ellison, Williams, 2012; Bettencourt da Silva, Williams, 2015; Rampsey, Ellison, Rostron, 2019; JCGM 106, 2012; Williams, Magnusson, 2021). In the pharmaceutical industry, decisions regarding the production and release of medicines are often based on measurements of potency, purity, and stability. Measurement uncertainty can be used to assess the risk of false conformity decisions to avoid incorrect conclusions about the quality, efficacy, and safety of the medicine (Weitzel, Johnson, 2012; Weitzel, 2012; Separovic *et al*., 2018; Separovic *et al*., 2023).

For example, if a measurement of a medicine's potency falls within the specification limits, it may be considered acceptable. However, if measurement uncertainty is not taken into account, the actual value may be out-of-specification limits, leading to the acceptance of a substandard, ineffective, and unsafe medicine (Williams, Magnusson, 2021; Weitzel, Johnson, 2012).

The importance of considering measurement uncertainty in the pharmaceutical industry should be emphasized, as it can avoid (or minimize the risk of) serious consequences for public health and safety. False conformity decisions can lead to the release of substandard, ineffective, and unsafe medicines, which can result in treatment failures and harm patients. Furthermore, incorrect decisions regarding the release of medicines can damage a company's reputation and financial stability (Kuselman *et al*., 2017a; Kuselman *et al*., 2017b; Pennecchi *et al*., 2018; Bettencourt da Silva *et al*., 2019; de Oliveira, Lourenço, 2021; Bettencourt da Silva *et al*., 2022; da Silva, Lourenço, 2023).

The risk of false decisions due to measurement uncertainty is a function of several factors, including the measured values and their uncertainties (JCGM 106, 2012; Williams, Magnusson, 2021; Kuselman *et al*., 2017a; Kuselman *et al*., 2017b; Pennecchi *et al*., 2018; Bettencourt da Silva *et al*., 2019; de Oliveira, Lourenço, 2021; Bettencourt da Silva *et al*., 2022; da Silva, Lourenço, 2023). If the measured value is far from the specification limits, then even a large measurement uncertainty may not result in a false decision. However, if the measured value is close to the specification limits, then even a small measurement uncertainty could result in a false decision (Kuselman *et al*., 2017a; Kuselman *et al*., 2017b; Pennecchi *et al*., 2018; Bettencourt da Silva *et al*., 2019; de Oliveira, Lourenço, 2021; Bettencourt da Silva *et al*., 2022; da Silva, Lourenço, 2023).

When measurements are correlated, their uncertainties can interact in complex ways that can impact the total risk of false decisions. Correlations between measurements can arise from a variety of sources, including shared sources of measurement error (e.g., metrological correlation). In some cases, correlations between measured values can reduce the total risk of false decisions. However, correlation between measurements can also increase the total risk of false decisions (Lourenço, Bettencourt da Silva, 2019; Separovic *et al*., 2019; Separovic *et al*., 2021).

To minimize the risk of false conformity decisions due to measurement uncertainty, the sources of uncertainty must be understood, and appropriate measures taken to reduce it. This can include the selection of appropriate measurement methods, proper equipment calibration, and the use of validated procedures. In addition, it is important to properly assess measurement uncertainty and provide appropriate training and education to personnel involved in making decisions based on measurements (Ellison, Williams, 2012; Bettencourt da Silva, Williams, 2015; Rampsey, Ellison, Rostron, 2019; JCGM 106, 2012; Williams, Magnusson, 2021; Separovic *et al*., 2023).

Decision rules for conformity assessment using measurement uncertainty information refer to the criteria used to evaluate the measurement results and determine if they meet specific requirements or standards (JCGM 106, 2012; Williams, Magnusson, 2021). Some common decision rules used in conformity assessment are 1) simple decision rule (shared risk), where the measurement result is

compared to the specification limits, and if it falls within the limits, the result is considered compliant; otherwise, it is considered non-compliant. 2) decision rules that take into account measurement uncertainty information: 2a) Pass/fail decision rule with the use of guard-bands, in which an acceptance interval is defined based on the specification limits and a guard-band width (multiple of measurement uncertainty for an appropriate confidence level), and the measured value is compared to this interval. If the measured value falls within the acceptance interval, the result is considered compliant; otherwise, it is considered non-compliant. 2b) Risk assessment considers both the measured value and measurement uncertainty to determine the likelihood of a compliant (or a non-compliant) decision. In other words, the risk value is estimated to decide if the result is compliant or not (JCGM 106, 2012; Williams, Magnusson, 2021).

Considering the decision rules previously described, a medicine with the active pharmaceutical ingredient (API) content of 92.0 ± 3.0% should be accepted according to the simple acceptance rule (assuming a regulatory specification limit from 90.0 to 110.0% of API content). However, it will be rejected according to the guard-band (assuming an acceptance interval from 92.5 to 107.5% of API content) and risk assessment rules (there will be an increased risk of false conformity decision – above 5%).

In this paper, we discussed how the measurement result (measured value and its uncertainty) and the selection of the decision rule impact the conformity assessment of medicines. The simple decision rule, pass/fail decision rule using guard-bands, and risk assessment were applied in multiparameter evaluations with correlated (ciprofloxacin ophthalmic solution medicines) and uncorrelated (acyclovir topical cream) measured values.

MATERIAL AND METHODS

Medicines samples and reference substances

Ciprofloxacin ophthalmic solutions from two different manufacturers (Lab A and Lab B) were purchased on the Brazilian market. In addition, acyclovir topical creams from three different manufacturers (Lab A, Lab B, and Lab C) were also purchased on the Brazilian market. Ciprofloxacin and acyclovir certified reference substances (CRS) were obtained from the United States Pharmacopeia (United States Pharmacopeia, 2021).

Pharmaceutical analysis

Ciprofloxacin ophthalmic solution analysis

Ciprofloxacin ophthalmic solution samples were subject to volume measurements, pH determination, density determination, assay for ciprofloxacin content, potency of ciprofloxacin, and drop test (Farmacopeia Brasileira, 2019).

Volume measurements were performed in 10 individual flasks using a calibrated volume apparatus. The pH determinations were performed using a pHmeter (PG1800, Gehaka) and certified reference buffers with pH of 4.0 and 7.0 for instrument calibration. Density determinations were performed with a calibrated pycnometer and a calibrated analytical balance (AUY220, Shimadzu).

Assay for ciprofloxacin content utilized a high-performance liquid chromatograph (Thermo, Accela) equipped with an octadecylsilane (C18 250 mm × 4 mm, 3–10 μm) column and with a UV detector (UV) adjusted to 280 nm. The mobile phase contained a mixture of 0.005 M tetrabutylammonium phosphate solution and methanol (75:25 v:v), with a flow rate of 1.5 mL/min. The samples and reference standard substance were diluted to a 0.12 mg/mL concentration using purified water as a diluent. Volumes of 20 μL of sample and standard solutions were injected, and the peak area measurements were used to calculate the amount of ciprofloxacin in the sample solution.

The potency of ciprofloxacin was verified using an agar diffusion microbiological assay. Petri dishes were prepared using 20 and 5 mL of antibiotic medium 11 as base and seed layer. The seed layer was inoculated with 1% *Staphylococcus epidermidis* (ATCC 12228) suspensions with a transmittance adjusted to 25 ± 2% at 580 nm. Sample and reference standard substance were diluted to a concentration of 2, 4, and 8 μg/mL using 0.1 M phosphate buffer as diluent. After incubation at 37 ± 1 °C for 18–24 h (Nova Ética), inhibition zone sizes were

measured using a zone reader (haloes caliper, IUL), and the potency of the sample solution was calculated.

The drop test was performed in 10 individual flasks to assess the ciprofloxacin content per drop. First, the weight of 10 drops was measured for each flask. Considering a density determination, the volume of each drop was calculated. Finally, the amount of ciprofloxacin per drop was calculated considering the volume per drop and the assay for ciprofloxacin content (HPLC).

All tests and assays were performed using both United States Pharmacopeia and Brazilian Pharmacopeia (Farmacopeia Brasileira, 2019).

Acyclovir topical cream analysis

Acyclovir topical cream samples were subject to weight measurements, bacterial and fungal enumeration tests (microbial counts), and an assay for acyclovir content (Farmacopeia Brasileira, 2019).

Weight measurements were performed in 10 individual units using a calibrated analytical balance (AUY220, Shimadzu).

Bacterial and fungal enumeration tests were performed by the pour plate method. Aliquots of 10 g of acyclovir topical cream samples were subject to decimal serial dilutions (1:10, 1:100, and 1:1000) using sterile 0.9% (w/v) sodium chloride solution. Aliquots of 1 mL of each dilution (1:10, 1:100, and 1:1000) were transferred to Petri dishes, and 15–20 mL of tryptic soy agar (TSA, BD) and Sabouraud dextrose agar (SDA, BD) culture media were placed for bacterial and fungal counts, respectively. Petri dishes containing TSA were incubated at 30–35 °C for 48–72 h (Nova Ética). Likewise, Petri dishes containing SDA were incubated at 20–25 °C for 5–7 days (Fanen incubator). The colony forming units (CFU) per plate were counted, and the microbial load of samples (CFU/g) was calculated considering appropriate dilution factors.

An assay for acyclovir content was performed using a UV spectrophotometer (Genesys 50, Thermo). Samples were subject to liquid-liquid extraction using ethyl acetate and 0.5 M sulfuric acid. Samples and reference standard substance were diluted to 15 µg/mL using purified water as diluent. The absorbances of the sample and standard solutions were measured at 255 nm, using 0.1 M sulfuric acid as blank. The amount of ciprofloxacin in the sample solution was calculated from the absorbance measurements.

All tests and assays were performed using both United States Pharmacopeia and Brazilian Pharmacopeia (Farmacopeia Brasileira, 2019).

Measurement uncertainty evaluation

Measurement uncertainty evaluations of volume, weight, pH, and density determinations were performed according to the law of uncertainty propagation (Ellison, Williams, 2012; Separovic *et al.*, 2023), considering the repeatability measurements and the uncertainties from the calibration certificate of instruments (pHmeter, volumetric apparatus, and analytical balance).

Uncertainty from bacterial and fungal enumeration tests were performed using a bottom-up approach, considering the uncertainty from sample weight, dilution factors, and the repeatability measurements of microbial counts (Hibbert, 2003; Dias, Lourenço, 2020; Dias, Lourenço, 2021). Microbial counts and the respective specification limits were log transformed to ensure a symmetric distribution (approximately normal distribution after log transformation).

The variability of inhibition zone sizes was the main source of uncertainty considered to assess the measurement uncertainty of ciprofloxacin potency estimated by the agar diffusion method (Saviano, Bettencourt da Silva, Lourenço, 2019). Although the uncertainty of the potency was estimated as a multiplicative factor, we assumed the measured value has an approximately normal distribution, since relative uncertainty is low (below 10%).

Measurement uncertainty associated with ciprofloxacin and acyclovir content was evaluated using bottom-up and/or top-down approaches (Separovic *et al.*, 2023; Ellison, 2005; Milde *et al.*, 2020; Morgado *et al.*, 2021; Morgado *et al.*, 2022; Pluháček *et al.*, 2023). For the top-down approach, two main sources of uncertainty were considered: 1) the trueness component assessed as the mean recovery of samples with known concentrations of ciprofloxacin and acyclovir; and 2) the precision component assessed as the standard deviation of

samples analyzed in repeatability conditions (Separovic *et al.*, 2018; Separovic *et al.*, 2023; Milde *et al.*, 2020).

Finally, the uncertainty associated with the drop test results was performed using the spreadsheet method (Separovic *et al.*, 2019; Ellison, 2005). The drop test results were calculated as a function of the density determination and the assay for ciprofloxacin content; therefore, the metrological correlation is not expected to be negligible (Lourenço, Bettencourt da Silva, 2019; Separovic *et al.*, 2019; Separovic *et al.*, 2021).

Multivariate guard-bands and total risk assessment

The widths of guard-bands (g) were calculated as the standard uncertainty (u) multiplied by an appropriate coverage factor (k). The guard-bands were summed and/or subtracted to the lower and/or upper specification limits ($LSL + g$ and/or $USL - g$), to obtain an acceptance zone that ensures an increased probability of correct acceptance (i.e., a reduced consumer's risk) (Lombardo, da Silva, Lourenço, 2022; da Silva, Lourenço, 2023).

Although the guard-bands ensure a reduced risk of false decision for a particular test (or parameters), the total risk of false decision may be unacceptable. Thus, the multivariate guard-bands were also calculated to ensure a reduced total risk of false decisions. Likewise, in conventional guard-bands, the widths of multivariate guard-band (g') were calculated as the standard uncertainty (u) multiplied by an appropriate multivariate coverage factor (k') (da Silva, Lourenço, 2023). Multivariate coverage factor (k') values were defined using the Monte Carlo method and MS-Excel Goal-Seek tool, implemented in an MS Excel worksheet (da Silva, Lourenço, 2023). Since the multivariate coverage factor may be affected by metrological correlation, the Monte Carlo method was adopted as it allowed to be defined using a numerical solution.

Moreover, the particular and total risk values were estimated using the Monte Carlo method. The simulated values were obtained using a normally distributed random generator, using the formula "=NORM.INV(RAND();x_i,u_{xi})", where x_i and u_{xi} are the measured value and its respective standard uncertainty for the i-th parameter (da Silva, Lourenço, 2023). The spreadsheet allows one to simulate correlated or uncorrelated simulated values because the metrological correlation may not be negligible. The total risk values were calculated as the number of simulated values out-of-specification limits for at least one of the tests (or parameters) divided by the total number of simulations (typically 50,000 simulations) (Separovic *et al.*, 2018; Kuselman *et al.*, 2017a; Kuselman *et al.*, 2017b; Pennecchi *et al.*, 2018). The MS Excel spreadsheet *Total Risk & Multivariate Guard-Bands.xlsm* is available in the supplementary material.

RESULTS AND DISCUSSION

Pharmaceutical analysis of ciprofloxacin ophthalmic solution

The results of volume measurements, pH determination, density determination, assay, potency, and drop test for ciprofloxacin ophthalmic solution samples are summarized in Table I.

TABLE I - Measured values and their standard uncertainties, specification limits, acceptance limits (univariate guard-band obtained using $k = 1.64$), multivariate acceptance limits (multivariate guard-band obtained using $k' = 2.35$), and risk assessment (consumer's risk values) for ciprofloxacin ophthalmic solution medicines from Lab A (generic) and B (reference)

Lab A (Generic medicine)	**Measured value and its standard uncertainties**	**Specification limits**	**Acceptance limits (Univariate guard-bands)**	**Multivariate acceptance limits (Multivariate guard-bands)**	**Risk assessment (consumers' risk values)**
Volume (mL)	5.2 ± 0.1	Min. 5.0	Min. 5.16	Min. 5.23	2.25 %
pH	4.5 ± 0.2	3.5 to 5.5	3.83 to 5.17	3.97 to 5.03	0.00 %
Density (g/mL)	1.015 ± 0.002	1.000 to 1.020	1.003 to 1.017	1.004 to 1.016	0.51 %
Assay (mg/mL)	3.28 ± 0.04	2.70 to 3.30	2.77 to 3.23	2.79 to 3.21	30.72 %
Potency (%)	97.4 ± 3.5 %	90.0 to 110.0	95.8 to 104.2	98.2 to 101.8	1.79 %
Drop Test (mg/drop)	119 ± 4	95 to 123	102 to 116	104 to 114	16.01 %
TOTAL					40.78 %
Lab B (Reference medicine)	**Measured value and its standard uncertainties**	**Specification limits**	**Acceptance limits (Univariate guard-bands)**	**Multivariate acceptance limits (Multivariate guard-bands)**	**Risk of false decision (consumers' risk values)**
Volume (mL)	5.5 ± 0.1	Min. 5.0	Min. 5.16	Min. 5.23	0.00 %
pH	4.4 ± 0.2	3.5 to 5.5	3.83 to 5.17	3.97 to 5.03	0.00 %
Density (g/mL)	1.005 ± 0.002	1.000 to 1.020	1.003 to 1.017	1.004 to 1.016	0.43 %
Assay (mg/mL)	3.26 ± 0.05	2.70 to 3.30	2.78 to 3.22	2.82 to 3.18	21.17 %
Potency (%)	100.4 ± 3.5 %	90.0 to 110.0	95.8 to 104.2	98.2 to 101.8	0.46 %
Drop Test (mg/drop)	177 ± 6	142 to 185	152 to 175	156 to 171	9.12 %
TOTAL					26.36 %

The pharmacopeia compendia usually adopted a simple acceptance rule (also called the shared risk rule). According to the simple acceptance rule, all the results obtained for ciprofloxacin ophthalmic solution medicines are within the specification limits (Figure 1, for generic medicine). However, the simple decision rule does not take into account the information of measurement uncertainty. Therefore, the risk of a false decision may be significantly high (up to 50%) (JCGM 106, 2012; Williams, Magnusson, 2021).

A decision rule that takes into account the measurement uncertainty information shows an increased risk of false decision for the assay of ciprofloxacin content and drop test for both Lab A (generic) and Lab B (reference) medicines. Thus, considering a guard-band decision rule, both Lab A (generic) and Lab B (reference) medicines should be rejected, because the assay of ciprofloxacin content and drop test results are out of the acceptance interval (see Table I).

Moreover, the consumers' risk values for the assay and drop test were 30.72% and 16.01%, respectively (Figure 1D and Figure 1F, for assay and drop tests of generic medicine – Lab A). Likewise, the consumers' risk values in the assay and drop test were 21.17% and 9.12%,

respectively, for Lab B (reference). The risk values were estimated by the Monte Carlo method using an MS Excel spreadsheet (*Total Risk & Multivariate Guard-Bands. xlsm*) available as supplementary material.

Estimating risk values may be laborious and complex for routine analysis. Thus, a pass/fail decision rule using guard-bands may be a simpler way for conformity/non-conformity assessment (JCGM 106, 2012; Williams, Magnusson, 2021; Separovic *et al.*, 2023). The guard-band (g) is defined as the standard uncertainty (u) multiplied by an appropriate coverage factor (k, typically, $k = 1.64$ for a 95% confidence level, or a 5% risk of false decision). The guard-band width is summed and/or subtracted to the lower and/or upper specification limits ($LSL + g$ and/or $USL - g$) to obtain an acceptance zone that ensures a reduced consumer's risk (JCGM 106, 2012; Williams, Magnusson, 2021; Separovic *et al.*, 2023). The acceptance limits for volume, pH, density, assay, potency, and drop tests are provided in Table I. The measured values for assay and drop tests were out of the acceptance zone, which is in accordance with the risk values previously discussed (see Table I).

Even if the measured values are within the acceptance limits, the total risk value may be significantly high (Lombardo, da Silva, Lourenço, 2022; da Silva, Lourenço, 2023). This may occur since the conventional guard-bands are useful to ensure a reduced risk of false decisions for a particular test (or parameter); however, they cannot guarantee a reduced total risk of false decisions (Lombardo, da Silva, Lourenço, 2022; da Silva, Lourenço, 2023). Thus, we proposed the calculation of multivariate guard-bands, which can reduce both the particular and total risks of false decisions. The multivariate guard-band (g') is defined as the standard uncertainty (u) multiplied by an appropriate multivariate coverage factor (k') (Lombardo, da Silva, Lourenço, 2022; da Silva, Lourenço, 2023). The k' value depends on the number of tests (or parameters) to be assessed and the correlation between them (e.g., metrological correlation between measured values due to sharing relevant analytical steps). A table with several k' values for 2 to 8 tests (or parameters) assessed, considering difference correlation scenarios (from uncorrelated to highly correlated values), was provided in da Silva, Lourenço (2023).

The multivariate acceptance limits for volume, pH, density, assay, potency, and drop tests are presented in Table I. Multivariate guard-bands widths were calculated using a multivariate coverage factor (k') of 2.35. Moreover, the metrological correlation due to sharing relevant analytical steps was considered. In the metrological correlation between assay and drop test values (Figure 2D, for generic medicine), the measured values for both assay and drop tests are out of the multivariate acceptance limits (Figure 1D and Figure 1F, for assay and drop tests of generic medicine), which is in accordance with the total risk values (40.78 and 26.36% for generic and reference medicines, respectively).

The k' value used to ensure a reduced total risk of a false decision may lead to narrow acceptance limits. For example, the multivariate guard-band provided a narrower acceptance interval (2.79 to 3.21 for ciprofloxacin assay) than the univariate (conventional) guard-band acceptance interval (2.77 to 3.23 mg/mL). This limitation may be overcome by reducing measurement uncertainty.

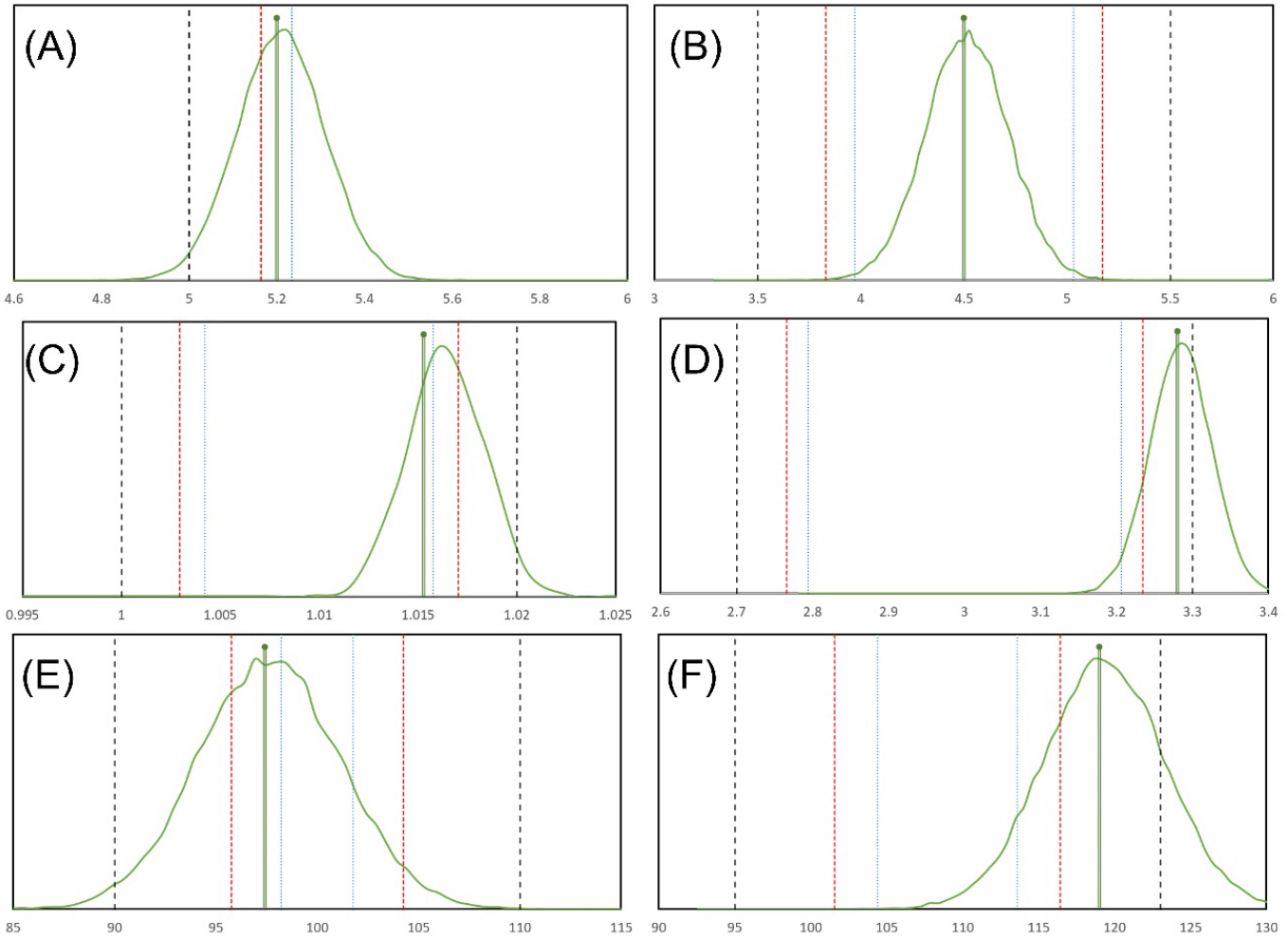

FIGURE 1 - Measured values (green lines), specification limits (black lines), acceptance limits (red lines), and multivariate acceptance limits (blue lines) for ciprofloxacin ophthalmic solution (generic medicine). Legend: (A) volume (mL); (B) pH; (C) density (g/mL); (D) assay (mg/mL); (E) potency (%); and (F) drop test (mg/drop).

The measured value and its respective measurement uncertainty (histogram), the specification limits, acceptance zones (univariate guard-bands), and multivariate acceptance zones (multivariate guard-bands) for volume (A), pH (B), density (C), assay (D), potency (E), and drop test (F) for ciprofloxacin ophthalmic solution generic medicine are presented in Figure 1.

Moreover, scatterplot graphs of density *vs.* assay (A), assay *vs.* potency (B), density *vs.* potency (C), assay *vs.* drop test (D), density *vs.* drop test (E), and potency *vs.* drop test (F) for ciprofloxacin ophthalmic solution generic medicine are presented in Figure 2. The correlation between assay and drop test values is not negligible (= 0.4073 and 0.4445 for generic and reference medicines, respectively) (Figure 2D, for generic medicine) and, consequently, may impact the total risk value and/or the multivariate coverage factor (*k'*).

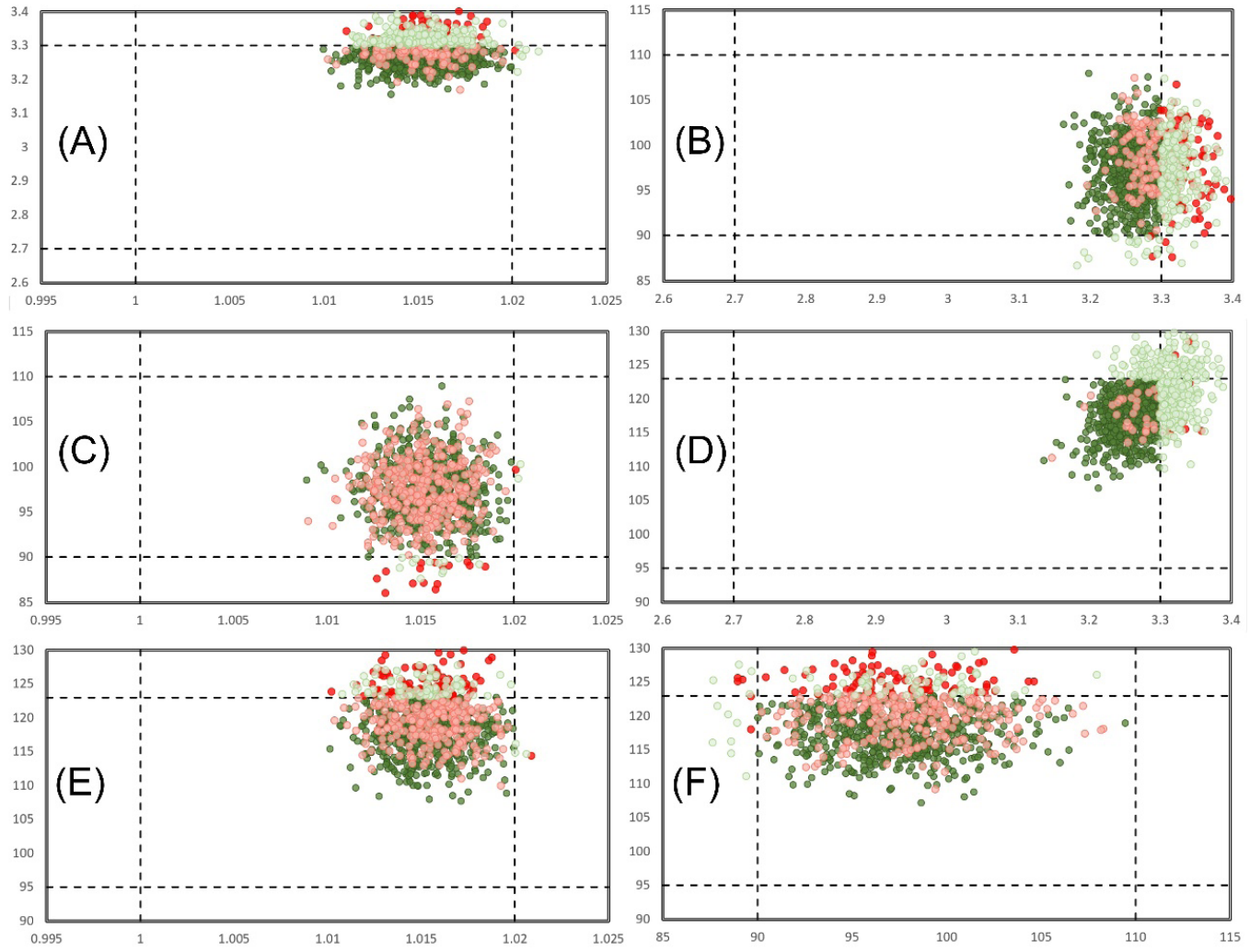

FIGURE 2 - Simulated values (dots) and specification limits (black lines) for ciprofloxacin ophthalmic solution (generic medicine). Legend: (A) density (g/mL) *vs.* assay (mg/mL); (B) assay (mg/mL) *vs.* potency (%); (C) density (g/mL) *vs.* potency (%); (D) assay (mg/mL) *vs.* drop test (mg/drop); (E) density (g/mL) *vs.* drop test (mg/drop); and (F) potency (%) *vs.* drop test (mg/drop). Dark green dots indicated simulated values within the specification for all tests. Light green dots indicate simulated values out-of-specification for at least one of the tests shown in the scatterplot but within the specification limits for all the other tests. Light red dots indicate simulated values within the specification limits for both tests in scatterplot but out-of-specification of at least one of the other tests. Dark red dots indicate simulated values out-of-specification for at least one of the tests in the scatterplot and out-of-specification for at least one of the other tests.

Pharmaceutical analysis of acyclovir topical cream

The results of weight measurements, bacterial and fungal enumeration tests (microbial counts), and assay for acyclovir topical cream samples are presented in Table II.

TABLE II - Measured values and their standard uncertainties, specification limits, acceptance limits (univariate guard-band obtained using $k = 1.64$), multivariate acceptance limits (multivariate guard-band obtained using $k' = 2.04$), and risk assessment (consumer's risk values) for acyclovir topical cream medicines from Lab A (generic), B (similar), and C (reference)

Lab A (Generic medicine)	**Measured value and its standard uncertainty***	**Specification limits**	**Acceptance limits (Univariate guard-bands)**	**Multivariate acceptance limits (Multivariate guard-bands)**	**Risk of false decision (consumers' risk values)**
Weight (g)	10.56 ± 0.11	Min. 10	10.17	10.22	0.000 %
Bacteria Count (CFU/g)	< 10	Max. 10^3	$10^{2.51}$	$10^{2.39}$	0.000 %
Fungal Count (CFU/g)	< 10	Max. 10^2	$10^{1.51}$	$10^{1.39}$	0.024 %
Assay (%)	104.8 ± 0.7	90.0 to 110.0	91.2 to 108.8	91.4 to 108.6	0.000 %
TOTAL					0.024 %
Lab B (Similar medicine)	**Measured value and its standard uncertainty***	**Specification limits**	**Acceptance limits (Univariate guard-bands)**	**Multivariate acceptance limits (Multivariate guard-bands)**	**Risk of false decision (consumers' risk values)**
Weight (g)	10.35 ± 0.10	Min. 10	10.17	10.21	0.030 %
Bacteria Count (CFU/g)	< 10	Max. 10^3	$10^{2.51}$	$10^{2.39}$	0.000 %
Fungal Count (CFU/g)	< 10	Max. 10^2	$10^{1.51}$	$10^{1.39}$	0.044 %
Assay (%)	98.3 ± 0.8	90.0 to 110.0	91.3 to 108.7	91.6 to 108.4	0.000 %
TOTAL					0.074 %
Lab C (Reference medicine)	**Measured value and its standard uncertainty***	**Specification limits**	**Acceptance limits (Univariate guard-bands)**	**Multivariate acceptance limits (Multivariate guard-bands)**	**Risk of false decision (consumers' risk values)**
Weight (g)	10.34 ± 0.10	Min. 10	10.17	10.21	0.036 %
Bacteria Count (CFU/g)	< 10	Max. 10^3	$10^{2.51}$	$10^{2.39}$	0.000 %
Fungal Count (CFU/g)	< 10	Max. 10^2	$10^{1.51}$	$10^{1.39}$	0.042 %
Assay (%)	102.1 ± 0.5	90.0 to 110.0	90.8 to 109.2	91.0 to 109.0	0.000 %
TOTAL					0.078 %

According to the simple acceptance rule adopted, all the results obtained for acyclovir topical cream medicines are within the specification limits (Figure 3, for generic medicine). When considering a decision rule that takes into account the measurement uncertainty information, the risks of false decision are acceptable for all tests of the three medicines (generic, similar, and reference medicines – from Lab A, Lab B, and Lab C, respectively), with risk

values below 5% (Table II). The risk values were estimated by the Monte Carlo method using the MS Excel spreadsheet (*Total Risk & Multivariate Guard-Bands.xlsm*) available as supplementary material. The total consumers' risk values found were 0.024%, 0.074%, and 0.078% for generic, similar, and reference medicines, respectively.

Adopting a pass/fail decision rule with the use of guard-bands clarifies that the measured values of all tests of the three medicines (generic, similar, and reference medicines – from Lab A, Lab B, and Lab C, respectively) were within the acceptance zone and multivariate acceptance zone, which ensures reduced particular and total risks of false conformity decisions (see Table II).

The measured value and its respective measurement uncertainty (histogram), specification limits, acceptance zones (univariate guard-bands), and multivariate acceptance zones (multivariate guard-bands) for weight (A), bacterial count (B), fungal count (C), and assay (D) for acyclovir topical cream generic medicine are presented in Figure 3.

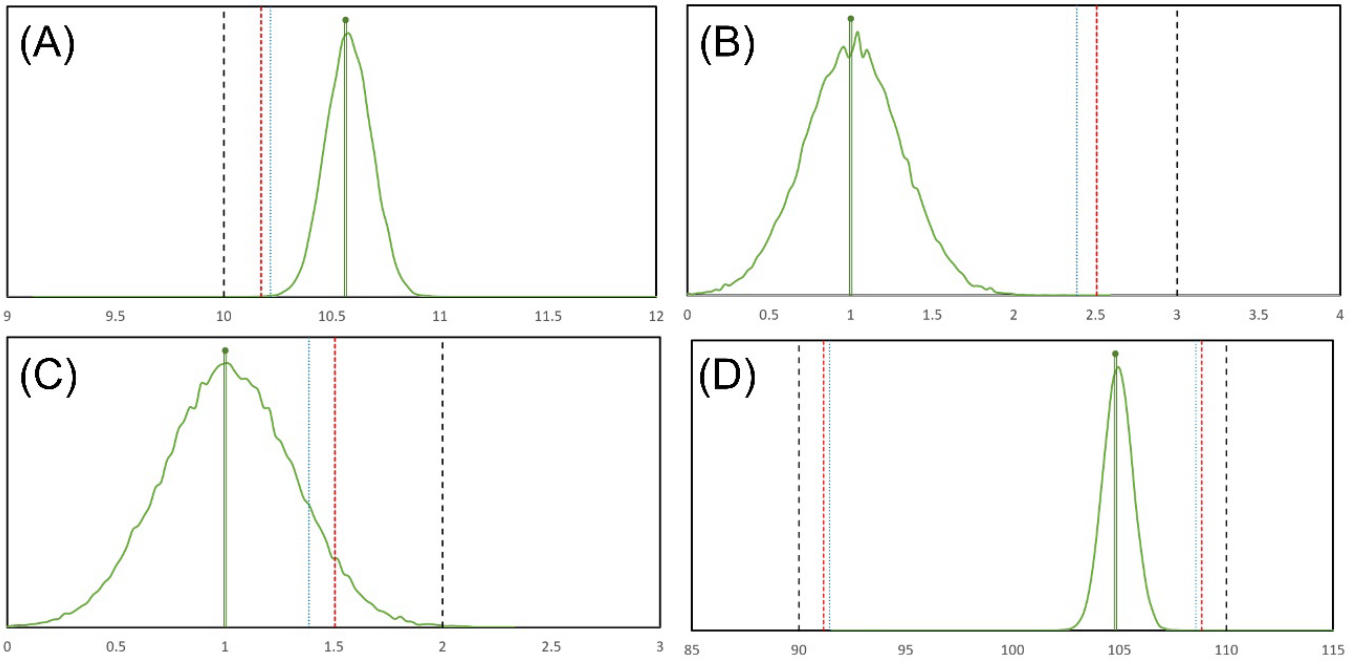

FIGURE 3 - Measured values (green lines), specification limits (black lines), acceptance limits (red lines), and multivariate acceptance limits (blue lines) for acyclovir topical cream (generic medicine). Legend: (A) weight (g); bacteria count (CFU/g); (C) fungal count (CFU/g); and (D) assay (%).

The acceptance limits (obtained using univariate guard-bands) and multivariate acceptance limits (obtained using multivariate guard-band) for weight, bacterial and fungal counts, and acyclovir assay are presented in Table II. Univariate and multivariate guard-band widths were calculated using a coverage factor (k) of 1.64 and a multivariate coverage factor (k') of 2.04. In the case of acyclovir topical cream analysis, we assumed that the metrological correlation between measured values is negligible since all tests were performed independently (without sharing relevant analytical steps) (see Figure 4).

Moreover, scatterplot graphs of weight *vs.* bacterial count (A), bacterial count *vs.* fungal count (B), weight *vs.* fungal count (C), bacterial count *vs.* assay (D), weight *vs.* assay (E), and fungal count *vs.* assay (F) for acyclovir topical cream generic medicine are presented in Figure 4. The scatterplot graphs indicate that the measured values are all uncorrelated (Figure 4, for generic medicine).

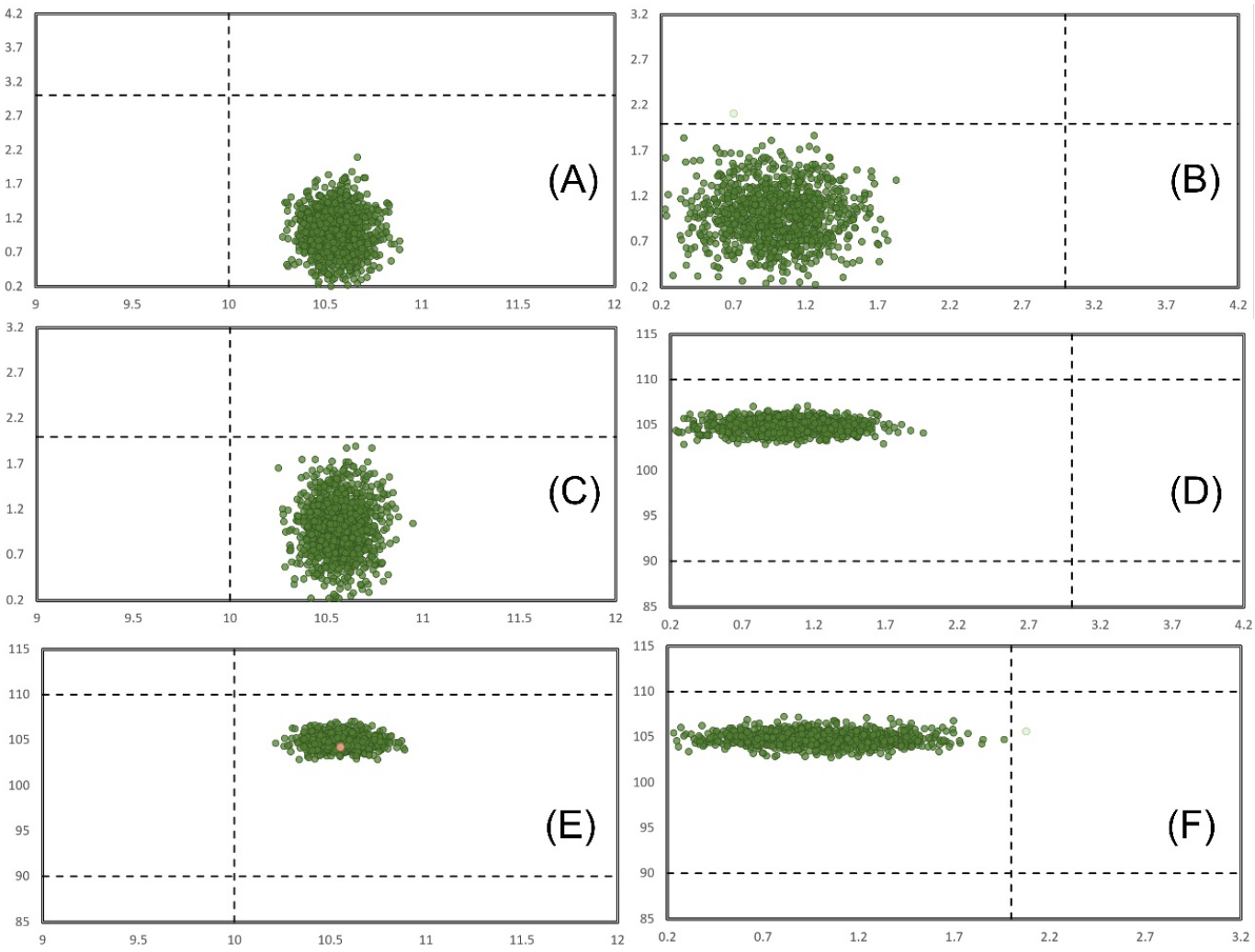

FIGURE 4 - Simulated values (dots) and specification limits (black lines) for acyclovir topical cream (generic medicine). Legend: (A) weight (g) *vs.* bacteria count (CFU/g); (B) bacteria count (CFU/g) *vs.* fungal count (CFU/g); (C) weight (g) *vs.* fungal count (CFU/g); (D) bacteria count (CFU/g) *vs.* assay (%); (E) weight (g) *vs.* assay (%); and (F) fungal count (CFU/g) *vs.* assay (%).

CONCLUSIONS

The simple acceptance rule usually adopted by pharmacopeial compendium is a simple decision rule; however, the risk of a false decision may be significantly high, particularly when the measured value is close to the specification limits and/or the measurement uncertainty is high. In contrast, decision rules that take into account measurement uncertainty information (pass/fail decision rule with the use of guard-bands and risk assessment) can control the risk of a false conformity decision.

Decisions made using simple acceptance rule and decision rules that consider measurement uncertainty (pass/fail decision rule with the use of guard-bands and risk assessment) may differ, as the first one does not consider the risk of a false decision. Therefore, the use of information of measurement uncertainty in conformity (non-conformity) assessment is highly recommended to ensure the proper efficacy, safety, and quality of medicines.

DECLARATION OF COMPETING INTEREST

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

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