

Risk of false conformity decisions due to measurement uncertainty of Active Pharmaceutical Ingredient: A multiparametric evaluation

Gustavo Kenji Toko Purcino do Amaral¹, Felipe Rebello Lourenço^{1*}

¹*Department of Pharmacy, School of Pharmaceutical Sciences,
University of Sao Paulo, Sao Paulo, Brazil*

Medicines must be subject to physical, chemical, and biological analysis to guarantee their quality, safety, and effectiveness. Despite the efforts to ensure the reliability of analytical results, some uncertainty will always be associated with the measured value, which can lead to false decisions regarding conformity/non-conformity assessment. This work aims to calculate the specific risk of false decisions regarding conformity/non-conformity of acetaminophen oral solution dosage form. The acetaminophen samples from five different manufacturers (A, B, C, D, and E) were subject to an active pharmaceutical ingredient assay, density test, and dose per drop test according to the official compendia. Based on measured values and their respective uncertainties, the risk values were calculated using the Monte Carlo method implemented in an MS Excel spreadsheet. The results for two acetaminophen oral solution samples (C and D) provided an increased total risk value of false acceptance (33.1% and 9.6% for C and D, respectively). On the other hand, the results for the other three acetaminophen samples (A, B, and E) provided a negligible risk of false acceptance (0.004%, 0.025%, and 0.045% for A, B, and E, respectively). This indicates that measurement uncertainty is very relevant when a conformity assessment is carried out, and information on the risks of false decisions is essential to ensure proper decisions.

Keywords: Conformity assessment. Consumer's risk. Measurement uncertainty. Monte Carlo simulation. Multiparametric evaluation. Risk of false decision.

INTRODUCTION

Before a drug is released for marketing, it must undergo analysis by the quality control sector. Such analyses evaluate the physical, chemical, and microbiological characteristics not only of the finished product but also of its packaging material, raw materials, and semi-finished product. In addition to being a regulatory requirement, this verification of compliance with the specifications is a necessary requirement to guarantee the safety, efficacy, and quality of the product (ICH Q8(R2), 2017; ICH Q9(R1), 2023; ICH Q10, 2015; ICH Q14, 2022).

The results must be expressed with their respective measurement uncertainty to be used for compliance assessment. When uncertainty is taken into account in the compliance assessment, four situations can occur (Figure 1): (I) The obtained result and its uncertainty are outside the specification limits; (II) The obtained result is outside the specification limit, but such limit is within the expanded uncertainty interval; (III) The obtained result is within the specification limit but such limit is within the expanded uncertainty interval; (IV) The obtained result and its uncertainty are within the specification limit (Bettencourt da Silva, Williams, 2015; Williams, Magnusson, 2021).

*Correspondence: F. R. Lourenço. Departamento de Farmácia. Faculdade de Ciências Farmacêuticas. Universidade de São Paulo. Av. Prof. Lineu Prestes, 580, CEP 05508-000, São Paulo, SP, Brasil. E-mail: feliperl@usp.br. ORCID: <https://orcid.org/0000-0002-2630-151X>. G. K. T. P. do Amaral, E-mail: gustavok74@gmail.com

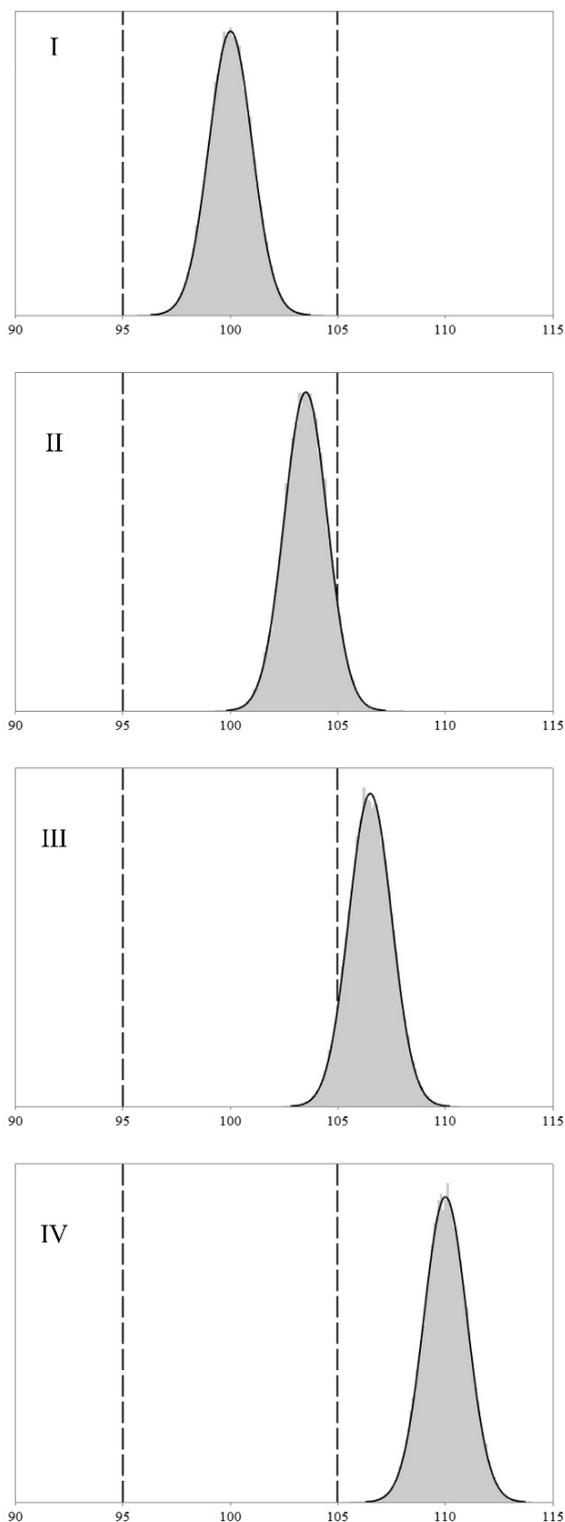


FIGURE 1 - Measured values and respective measurement uncertainties compared to the specification limits. (I) The obtained result and its uncertainty are outside the specification limits; (II) The obtained result is outside the specification limit, but such limit is within the expanded uncertainty interval; (III) The obtained result is within the specification limit, but such limit is within the expanded uncertainty interval; (IV) The obtained result and its

uncertainty are within the specification limit.

These situations lead to the risk of false compliance decisions, such as the acceptance of batch that is out of specification (consumer's risk) or the rejection of a batch that is actually within specification (consumer's risk) (Separovic, Lourenço, 2020; Bettencourt da Silva *et al.*, 2019; Pennechi *et al.*, 2018; Bettencourt da Silva *et al.*, 2018; Kuselman *et al.*, 2018; Kuselman *et al.*, 2017).

The risk of false compliance decision can have specific and global risks. The specific risk considers the result (measured value and respective measurement uncertainty) of a product or batch that has already been tested. The global risk corresponds to the false acceptance (or rejection) of a product or lot that will be tested based on prior knowledge (e.g., historical information of the manufacturing process). The specific risk is estimated using a frequentist approach, while the global risk is estimated using the Bayesian approach (Separovic, Lourenço, 2019; Bettencourt da Silva *et al.*, 2018).

Particular risk is the estimated probability of a false decision for a single quality parameter (e.g., content of active pharmaceutical ingredient in dosage form). However, even if the particular risk values are below the maximum admissible value (usually 5%), the total risk of false decisions may increase. Total risk is the probability of false decisions estimated for all parameters of quality simultaneously (e.g., content of active pharmaceutical ingredient in dosage form, density test, and dose per drop test, simultaneously) (Bettencourt da Silva *et al.*, 2019; Pennechi *et al.*, 2018; Bettencourt da Silva *et al.*, 2018; Kuselman *et al.*, 2018; Kuselman *et al.*, 2017; Separovic *et al.*, 2023). The use of measurement uncertainty information to support conformity/non-conformity decisions may also be applied in other areas, such as nuclear medicine facilities, water analysis, and fuels (Carvalho *et al.*, 2023; Brandão *et al.*, 2022; Matos, de Oliveira, 2021; de Oliveira, 2020).

Our aim was to analyze the results (measured values and their respective uncertainties) of an active pharmaceutical ingredient (API) assay (by UV spectrophotometry), density and dose per drop test of acetaminophen oral solution dosage forms from different manufacturers. We also estimated particular and total

specific risk values of false conformity decisions due to measurement uncertainty. Estimation of particular and total risk values is useful to support decision-making about the acceptance or rejection of a product or batch.

MATERIAL AND METHODS

Acetaminophen oral solution tests

Acetaminophen oral solution dosage forms obtained from five different manufacturers (medicines A, B, C, D, and E) were subjected to API assay (by UV spectrophotometry), density test, and dose per drop test. API assays were performed using a UV spectrophotometer (Thermo Scientific, Genesys 50), with absorbance measurements at 249 nm of sample solutions diluted in acidified methanol at $10 \mu\text{g mL}^{-1}$. Density measurements were performed using a calibrated pycnometer and an analytical balance (Shimadzu, AU220). The dose per drop test was performed using the average weight of 20 drops (from 10 different flasks), density, and API assay test results.

The results used to estimate particular and total risk values of false compliance decisions were obtained from previously published articles. The results of API assays (obtained from UV spectrophotometry), density tests, and dose per drop tests were obtained from Francisco *et al.* (2016) and Moreira and Lourenço (2015).

Particular and Total risk estimation

Particular and total risk values were estimated using Monte Carlo method (MCM), which was implemented using a Microsoft Excel spreadsheet. MCM was performed using 50,000 simulations for API assay, density, and dose per

drop test results, adopting a normally distributed random generator, with mean and standard deviation values that correspond to the measured value and standard uncertainty values, respectively. The Excel formula used to implement MCM was “=NORM.INV(RAND(); x_{ij} ; u_{xij})”, where x_{ij} and u_{xij} are the measured value and standard uncertainty for the i -th parameter (API assay, density test, or dose per drop test) of the j -th medicine (medicine A, B, C, D, or E).

The simulated values were compared to the specification limits, being classified as “accepted” or “rejected” (Farmacopeia Brasileira, 2019). Consumer’s and producer’s risk values were calculated using Eq.(1) and Eq.(2), respectively. Particular and total risk values were calculated using a frequentist approach (Separovic, Lourenço, 2019).

$$R_c = \frac{n_r}{N} \quad \text{Eq.(1)}$$

$$R_p = \frac{n_a}{N} \quad \text{Eq.(2)}$$

where, R_c and R_p are the consumer’s and producer’s risk values, respectively; n_r and n_a are the number of simulated values classified as “rejected” and “accepted”, respectively; and N is the total number of simulated values ($N = n_r + n_a$).

RESULTS AND DISCUSSION

A summary of the results (measured values and respective uncertainty values) of API assay, density test, dose per drop test obtained for acetaminophen oral solution from different manufacturers (medicines A, B, C, D, and E) are presented in Table I.

TABLE I - Measured values and respective uncertainty values of API assay, density test, and dose per drop test obtained for acetaminophen oral solution from different manufacturers (medicines A, B, C, D, and E)

Medicine	Assay (mg.mL^{-1})	Density (g.mL^{-1})	Dose per drop (mg.drop^{-1})
Specification limits	185.0 to 215.0	1.1400 to 1.1600	11.3 to 15.3
A (Reference)	210.6 ± 2.3	1.1490 ± 0.0036	13.2 ± 0.6
B (Generic)	210.9 ± 2.3	1.1530 ± 0.0036	13.7 ± 0.6

TABLE I - Measured values and respective uncertainty values of API assay, density test, and dose per drop test obtained for acetaminophen oral solution from different manufacturers (medicines A, B, C, D, and E)

Medicine	Assay (mg.mL ⁻¹)	Density (g.mL ⁻¹)	Dose per drop (mg.drop ⁻¹)
C (Generic)	214.5 ± 2.3	1.1500 ± 0.0036	13.6 ± 0.6
D (Generic)	213.4 ± 2.3	1.1440 ± 0.0036	13.9 ± 0.6
E (Generic)	206.0 ± 2.3	1.1460 ± 0.0036	13.0 ± 0.6

Measurement uncertainty values were expressed as expanded uncertainty ($k = 2$, for 95% confidence level).

Particular and total risk values were estimated using MCM, based in the results of API assay, density test, dose per drop test obtained for acetaminophen oral solution from different manufacturers (medicines A, B, C, D, and E). For each parameter of each sample, five MCM runs (using 100,000 simulated values) were performed and the consumer's or producer's risk values were expressed as the mean value of the five runs. To calculate the total specific risk, if at least one of the three

parameters (API assay, density test, or dose per drop test) were out-of-specification limits, then this simulated batch would be considered "rejected". On the other hand, the simulated batch would be considered "accepted" if all three parameters (API assay, density test, or dose per drop test) were within the specification limits. A summary of the particular and total consumer's risk values obtained for the batches from the different manufacturers is presented in Table II.

TABLE II - Particular and total consumer's risk values obtained assuming regulatory specification limits

Medicine	Particular risks for			Total risk
	API assay	Density	Dose per drop	
A (Reference)	0.004%	0.000%	0.000%	0.004%
B (Generic)	0.020%	0.005%	0.000%	0.025%
C (Generic)	33.1%	0.000%	0.000%	33.1%
D (Generic)	8.2%	1.3%	0.000%	9.6%
E (Generic)	0.000%	0.045%	0.000%	0.045%

Risk values were obtained using Monte Carlo method (50,000 simulated values for each test).

The results and respective risk values for API assay indicate an increased risk of false acceptance decision for medicines C (33.1%) and D (8.2%). On the other hand, the consumer's risk values for medicines A (0.004%), B (0.020%), and E (0.000%) are negligible. The increased risk values obtained for medicines C and D is because the measured values (214.5 and 213.4 mg.mL⁻¹ for medicines C and D, respectively) are close

to the specification upper limit (215.0 mg.mL⁻¹). The measurement uncertainty values (2.3 and 2.3 mg.mL⁻¹ for medicines C and D, respectively) are below the maximum admissible uncertainty value (target uncertainty) (3.75 mg.mL⁻¹) (Separovic, Bettencourt da Silva, Lourenço, 2019; Separovic, Bettencourt da Silva, Lourenço, 2021; Lourenço, Bettencourt da Silva, 2019).

In the results and respective risk values for the density test, the consumer’s risk value obtained for medicine D was 1.3%, which is below the maximum admissible risk value (5%). Moreover, the risk of false acceptance decision for all other medicines was negligible (0.000%, 0.005%, 0.000%, and 0.045% for medicines A, B, C, and E, respectively). Considering the results

and respective risk values for dose per drop test, the consumer’s risk values for all medicines were negligible (0.000% for medicines A, B, C, D, and E).

The histograms obtained for API assay, density test, and dose per drop test for the medicines from different manufacturers (medicines A, B, C, D, and E) are presented in Figure 2.

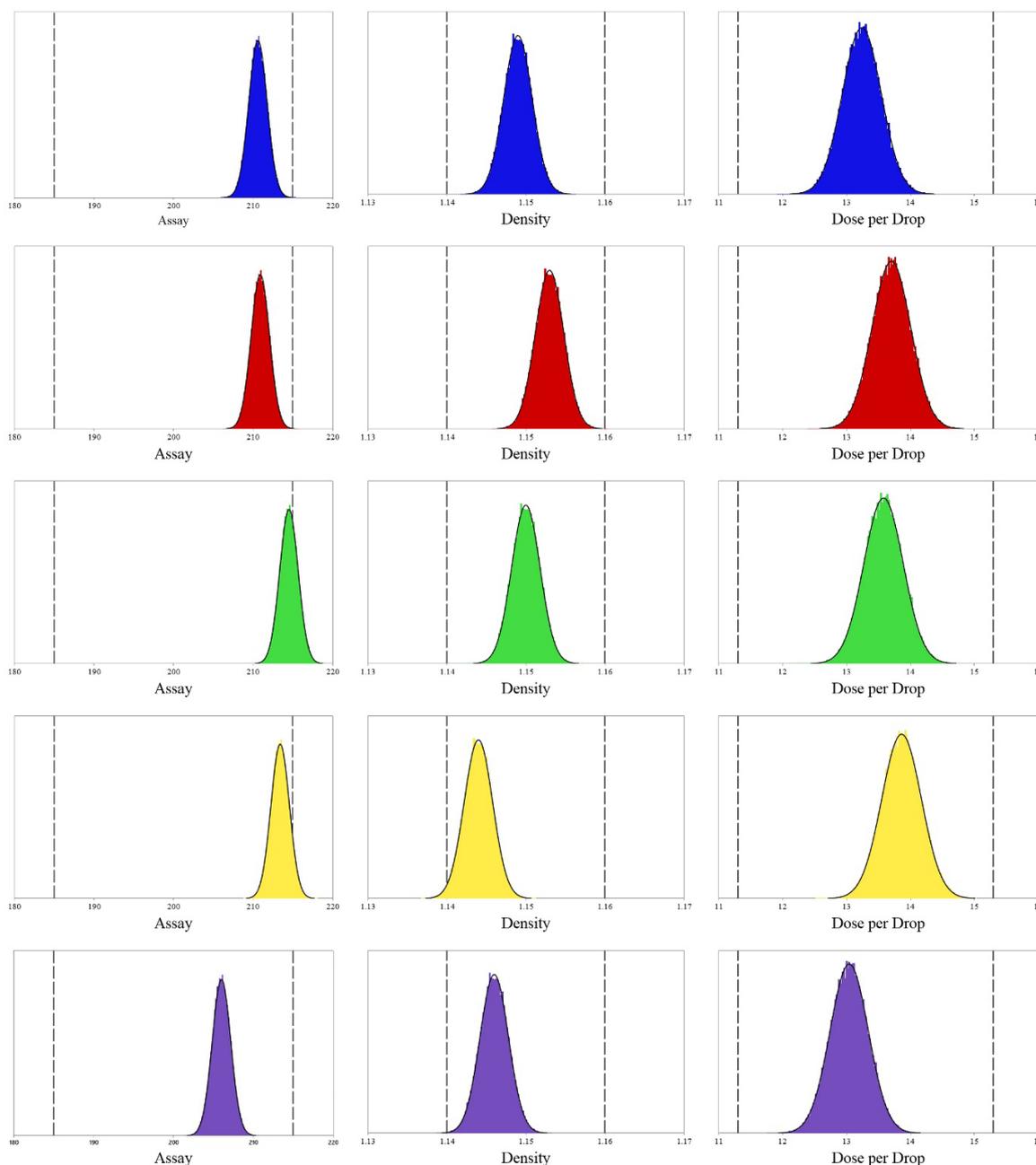


FIGURE 2 - Histograms obtained for API assay, density test, and dose per drop test for the medicines from different manufacturers. Legend: medicine A: blue histograms; medicine B: red histograms; medicine C: green histograms; medicine D: yellow medicines; and medicine E: purple histograms. Dot lines correspond to regulatory specification limits.

The particular risk values allow the risk assessment of false decisions for a single test or assay. However, even if the particular risk values are below the maximum admissible value (usually 5%), the total risk value may increase, because total risk considers the compliance assessment of all tests and assays simultaneously. The total consumer's risk value obtained for medicine C (33.1%) is mainly explained by the particular risk value of the API assay (33.1%). For medicine D, the total consumer's risk value (9.6%) was explained by both the particular risk values of API assay (8.2%) and the density test (1.3%). In both cases (medicines C and D), the total consumer's risks are above the maximum admissible value (5%), which may lead to false acceptance decisions. On the other hand, the total risk values obtained for medicines A, B, and E are negligible at 0.004%, 0.025%, and 0.0045%, respectively).

The total risk value may be impacted due to the correlation between measured values (Separovic, Bettencourt da Silva, Lourenço, 2019; Separovic, Bettencourt da Silva, Lourenço, 2021; Lourenço,

Bettencourt da Silva, 2019). Correlation between values may arise due to the characteristics of the products or batches tested (natural or intrinsic correlation) or due to the way measurements were performed (artificial or metrological correlation) (Separovic, Bettencourt da Silva, Lourenço, 2019; Separovic, Bettencourt da Silva, Lourenço, 2021; Lourenço, Bettencourt da Silva, 2019). The results of the dose per drop test are obtained sharing relevant analytical steps from the API assay and density test, thus metrological correlations between the parameters may not be negligible and, consequently, could affect the total risk values. To evaluate the impact of metrological correlation, the simulated measured values obtained for API assay, density test, and dose per drop test were plotted in dispersion plots. The dispersion plots for the medicines from different manufacturers are shown in Figure 3. For all medicines, the measured values of dose per drop test were highly correlated with the measured values of API assay. On the other hand, the correlations between measured values of API and density test and between measured values of density and dose per drop test were negligible (Figure 3).

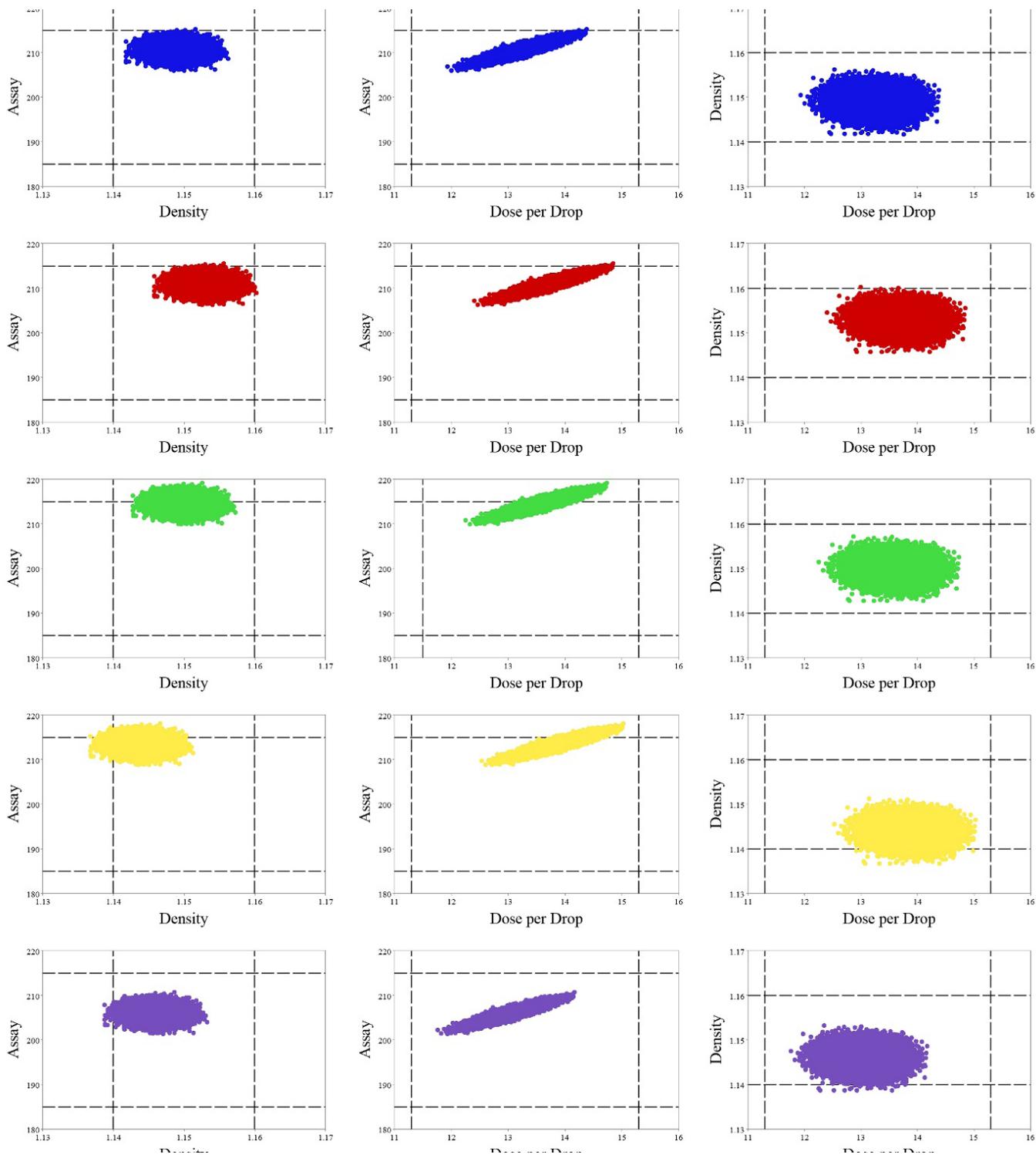


FIGURE 3 - Dispersion plots between measured values of API assay and density test, between measured values of API assay and dose per drop test, and between measured values of density and dose per drop tests for the medicines from different manufacturers. Legend: medicine A: blue histograms; medicine B: red histograms; medicine C: green histograms; medicine D: yellow medicines; and medicine E: purple histograms. Dot lines correspond to regulatory specification limits.

CONCLUSIONS

A measured value close to the specified limits and/or inappropriately high measurement uncertainty (e.g. above target measurement uncertainty), may indicate increased risk of false acceptance (consumer's risk) or rejection (producer's risk) decisions. In this work, total risk values were mainly due to the increased particular risk values for API assay results. However, even if particular risk values are below the maximum admissible risk, the increased risk of false compliance/non-compliance decisions may increase. The total risk may also be affected by the correlation between the measured values. In conclusion, measurement uncertainty provided relevant information and should be taken into account to support conformity/non-conformity decisions. Conformity decisions supported through a consumer's risk evaluation may reduce the probability of patients receiving substandard quality 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.

ACKNOWLEDGMENTS

This work was supported by FAPESP -Fundação de Amparo à Pesquisa do Estado de São Paulo (Brazil) (2022/08406-5) and CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico, Bolsista do CNPq – Brasil (304814/2022-9).

REFERENCES

- Bettencourt da Silva RJN, Lourenço FR, Pennechi FR, Hibbert DB, Kuselman I. Spreadsheet for evaluation of global risks in conformity assessment of a multicomponent material or object. *Chemom Intell Lab Syst.* 2019;188;1-5. <https://doi.org/10.1016/j.chemolab.2019.02.010>.
- Bettencourt da Silva RJN, Pennechi FR, Hibbert DB, Kuselman I. Tutorial and spreadsheets for Bayesian evaluation of risks of false decisions on conformity of a multicomponent material or object due to measurement uncertainty. *Chemom Intell Lab Syst.* 2018;182;109-16. <https://doi.org/10.1016/j.chemolab.2018.09.004>.
- Bettencourt da Silva RJN, Williams A. *Eurachem/CITAC Guide: Setting and Using Target Uncertainty in Chemical Measurement.* 2015. <https://eurachem.org>.
- Brandão LP, Silva V.F, Bassi M, de Oliveira EC. Risk assessment in monitoring of water analysis of a Brazilian river. *Molecules* 2022;27(11). <https://doi.org/10.3390/molecules27113628>.
- De Oliveira EC. Use of measurement uncertainty in compliance assessment with regulatory limits. *Braz J Anal Chem.* 2020;7(28):1-2. <http://dx.doi.org/10.30744/brjac.2179-3425.editorial.ecoliveira.N28>.
- Farmacopéia Brasileira. Vol. 2. Agência Nacional de Vigilância Sanitária (ANVISA). 2019.
- Francisco FL, Saviano AM, Lourenço FR. Measuring uncertainty using different approaches: A case study for acetaminophen quantification. *J AOAC Int.* 2016;99:612-617. <https://doi.org/10.5740/jaoacint.15-0199>.
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH guideline Q8 (R2) on pharmaceutical development. Q8(R2), 2017. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-11.pdf
- 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. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-17.pdf
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH guideline Q10 on pharmaceutical quality system. Q10, 2015. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human_en.pdf
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH guideline Q14 on analytical procedure development. Q14, 2022. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q14-analytical-procedure-development-step-2b_en.pdf
- Kuselman I, Pennechi F, da Silva, R, Hibbert D, Anchutina E. Total risk of a false decision on conformity of an alloy

due to measurement uncertainty and correlation of test results. *Talanta*. 2018;189:666-674. <https://doi.org/10.1016/j.talanta.2018.07.049>.

Kuselman I, Pennecchi FR, Bettencourt da Silva RJN, Hibbert DB. Risk of false decision on conformity of a multicomponent material when test results of the components' content are correlated. *Talanta*. 2017;174:789-796. <https://doi.org/10.1016/j.talanta.2017.06.073>.

Lourenço FR, Bettencourt da Silva RJN. Risk of false conformity decisions of multicomponent items controlled by correlated measurement results due to the sharing of analytical steps. *Talanta*. 2019;196:174-181. <https://doi.org/10.1016/j.talanta.2018.12.021>.

Matos ACH, de Oliveira EC. Risk assessment and optimisation of sulfur in marketing fuels. *Fuel*. 2021;122705. <https://doi.org/10.1016/j.fuel.2021.122705>.

Moreira CS, Lourenço FR. In Vitro Therapeutic Equivalence of Acetaminophen Oral Solution in Dripper Bottle. *Latin Am J Pharm*. 2015;34:78-85.

Pennecchi FR, Kuselman I, Bettencourt da Silva RJN, Hibbert DB. Risk of a false decision on conformity of an environmental compartment due to measurement uncertainty of concentrations of two or more pollutants. *Chemosphere*. 2018;202:165-76. <https://doi.org/10.1016/j.chemosphere.2018.03.054>.

Separovic L, Bettencourt da Silva RJN, Lourenço FR. Determination of intrinsic and metrological components of the correlation of multiparameter products for minimising the risks of false conformity decisions. *Measurement*. 2021;180:109531. <https://doi.org/10.1016/j.measurement.2021.109531>.

Separovic L, Bettencourt da Silva RJN, Lourenço FR. Improved spreadsheet method for determination of between components metrological correlation due to the sharing of analytical steps. *Chemom Intell Lab Sys*. 2019;89:161-168. <https://doi.org/10.1016/j.chemolab.2019.05.002>.

Separovic L, Lourenço FR. Frequentist approach for estimation of false decision risks in conformity assessment based on measurement uncertainty of liquid chromatography analytical procedures. *J Pharm Biomed Anal*. 2020;184:1-7. <https://doi.org/10.1016/j.jpba.2020.113203>.

Separovic L, Lourenço FR. Measurement uncertainty and risk of false conformity decision in the performance evaluation of liquid chromatography analytical procedures. *J Pharm Biomed Anal*. 2019;171:73-80. <https://doi.org/10.1016/j.jpba.2019.04.005>.

Separovic L, Simabukuro RS, Couto AR, Bertanha MLG, Dias FRS, Sano AY, et al. Measurement Uncertainty and Conformity Assessment Applied to Drug and Medicine Analyses – A Review. *Crit Rev Anal Chem*. 2023;53(1):123-138. <https://doi.org/10.1080/10408347.2021.1940086>.

Williams A, Magnusson B. Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment. 2021. <https://eurachem.org>.

Received for publication on 04th August 2023
Accepted for publication on 02nd October 2023