DETERMINATION OF RELATIVE POTENCY AS THE RATIO OF EFFECTIVE CONCENTRATION ESTIMATED FROM SIGMOIDAL-RESPONSE CURVES AND RESPECTIVE MEASUREMENT UNCERTAINTY

Felipe Rebello Lourenço[1*]

[1*]Department of Pharmacy/School of Pharmaceutical Sciences, University of São Paulo, Brazil, feliperl@usp.br

INTRODUCTION

Bioassays are *in vivo*, *ex vivo* or *in vitro* assays often used for the determination of the activity or relative potency (ρ) of drug products, which may be applied during process development, product development or product release testing. Bioassays are typically performed using a parallel-assay or a sigmoidal curve assay. Assuming that the standard (S) and test (T) samples are biologically similar, the test sample can be expected to behave like a dilution of the standard ($d_T = \rho \times d_S$). When sigmoidal curve assays are adopted, the relative potency is calculated as the ratio of effective concentration of test sample and standard ($\rho = EC50\%_T / EC50\%_S$).[1] The standard uncertainties of the effective concentrations of test ($u_{EC50\%_T}$) and standard ($u_{EC50\%_S}$) is used to calculate the combined uncertainty of the relative potency (u_ρ). However, $EC50\%_T$ and $EC50\%_S$ may be correlated due to shared relevant experimental conditions, which may affect the measurement uncertainty of the relative potency (u_ρ).[2] The aim of this work was to propose a methodology for the measurement uncertainty evaluation of the relative potency determined using a smartphone-based colorimetric assay and sigmoidal-response curves.

EXPERIMENTAL METHODS

Aliquots of standard and test samples in a range from 100 to 0.2 µg·mL^{-1} was transferred to a 96-well microtiter plate, followed by the addition of tryptic soy broth (TSB) previously inoculated with 10^5-10^6 CFU·mL^{-1} of *Staphylococcus aureus* (ATCC 6538 – 10^5-10^6 CFU·mL^{-1}) and resazurin solution. Microtiter plate was incubated at 37 ± 1ºC for 90 minutes. After incubation, the microbial growth inhibition was measured using a smartphone camera device and a colour analyser app (RGB - Red-Green-Blue).

RESULTS AND DISCUSSION

A 4-parameter logistic regression model was used to explain the microbial inhibition growth ($Y\%$) as function of the logarithm of the antibiotic ($ln(d)$), as presented in the equation below:

$$Y\% = A + \frac{(D - A)}{1 + e^{B \times (ln(C) - ln(d))}}$$

The upper and lower asymptotes (A and D, respectively), the slope (B) and the inflection point (C) are expected to be the same for both standard (S) and test (T) sigmoidal curves, since the standard and test samples are assumed to be to be biologically similar, as can be seen of **Figure 1**.

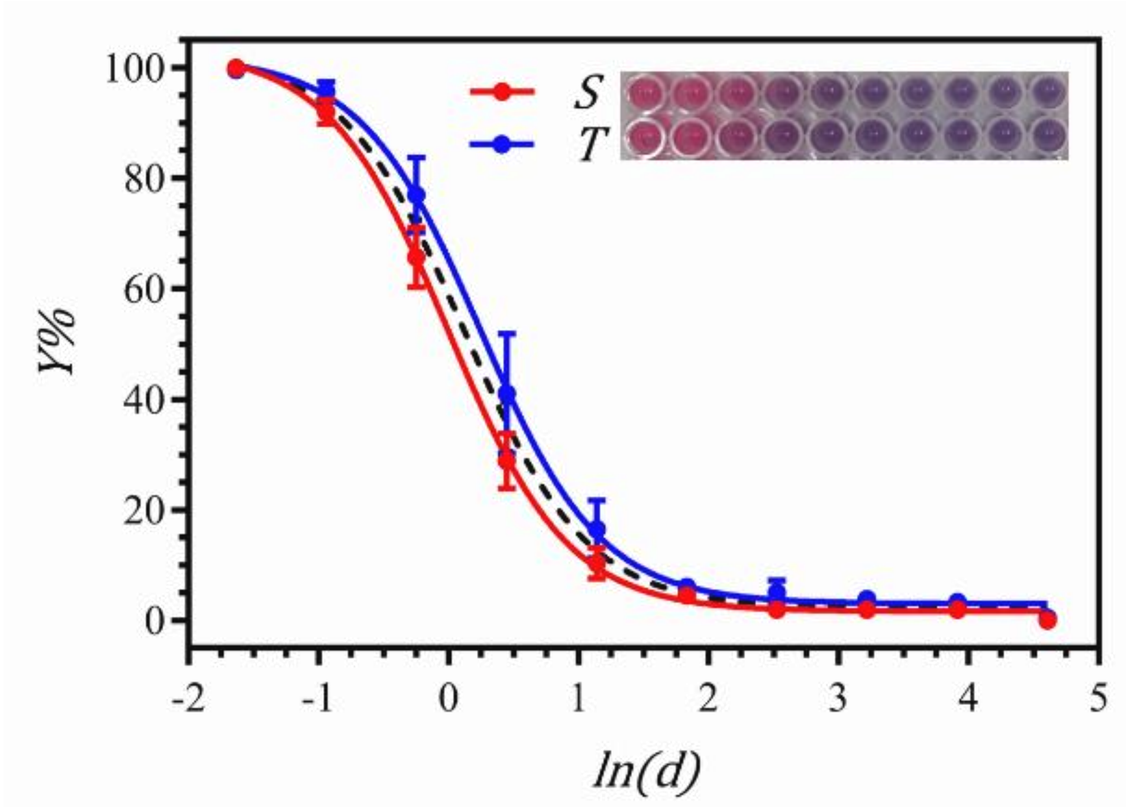

Figure 1. Sigmoidal curves for standard (S) and test (T) samples.

$EC50\%_T$ and $EC50\%_S$ values were found to be 1.18 and 1.16 µ·mL^{-1} and the respective uncertainty factors (U_F) were 1.065 and 1.061, respectively. In addition, $EC50\%_T$ and $EC50\%_S$ quantity values were significantly correlated (r = 0.83), due to shared experimental conditions. Kragten spreadsheet method[3] were used to estimate the measurement uncertainty associated with the relative potency calculate as the ratio of effective concentration of test sample and standard. Considering the correlation between $EC50\%_T$ and $EC50\%_S$ quantity values, the relative potency was found to be 98.5% *,/ 1.036. While the relative potency was found to be 98.5% *,/ 1.091, when the correlation between $EC50\%_T$ and $EC50\%_S$ quantity values were not considered. The target uncertainty factor (U_F^{target}) was found to be 1.052, considering the specification range from 90 to 135% for relative potency.

CONCLUSION

The correlation between $EC50\%_T$ and $EC50\%_S$ quantity values reduced significantly the uncertainty factor for relative potency, which is smaller than the target uncertainty factor.

REFERENCES

1. Saviano A.M., Bettencourt da Silva R.J.N., Lourenço F.R., *Regul. Toxicol. Pharmacol.* **2019**, *102*, 117-124.
2. Lourenço F.R.; Bettencourt da Silva R.J.N., *Talanta*. **2019**, *196*, 174-181.

ACKNOWLEDGMENTS

This work was supported by FAPESP (Brazil).

KEYWORDS

Bioassays, relative potency, measurement uncertainty, multiplicative uncertainty factor, correlation.