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Original Article

ESTIMATION OF *IN VIVO* PERFORMANCE OF SULFAMETHOXAZOLE AND TRIMETHOPRIM FROM ORAL SUSPENSIONS USING *IN VITRO* RELEASE DATA FROM A MINI PADDLE APPARATUS

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ABSTRACT

Objective: To estimate plasma concentrations-time profiles of sulfamethoxazole (SMZ) and trimethoprim (TMP) from oral pediatric suspensions through *in vitro* data generated with a mini paddle apparatus and dissolution media of physiological relevance. Post-marketing evaluation of pediatric formulations is always necessary.

Methods: Dissolution profiles of SMZ/TMP were obtained with a mini paddle apparatus at 100 rpm and 200 ml of 0.1 N HCl (pH 1.2), pH 4.5 acetate buffer, and pH 6.8 phosphate buffer. The reference and three multi-source pediatric formulations were tested. Drugs were quantified by a UV derivative method. Dissolution profiles were compared with model-independent and model-dependent methods. Plasma levels were estimated with dissolution data and published *in vivo* information. Percent of prediction error (%PE) for C_{max} and $AUC_{0\text{-}inf}$ at each condition was calculated.

Results: In all conditions, similar dissolution profiles were found excepting for TMP of C drug product at pH 1.2 (f_2<50). With model-independent comparisons significant differences in *in vitro* release performance of SMZ and TMP from all multi-source formulations were found (*P<0.05). When comparing the hypothetical C_{max} and $AUC_{0\text{-}inf}$ of both drugs with *in vivo* data PE<15% were found only with reference and one formulation at pH 1.2.

Conclusion: The mini paddle apparatus and dissolution media of pH 1.2 were the best conditions to estimate *in vivo* plasma concentrations of SMZ and TMP from reference. These settings seem adequate to evaluate *in vitro* performance of multi-source formulations. It is necessary to carried out human studies with the used fixed-dose combination formulations to correlate *in vitro*/*in vivo* data.

Keywords: Convolution, Sulfamethoxazole, Suspensions, Trimethoprim, Prediction Error

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INTRODUCTION

Children under seven years old are unable to swallow capsules or tablets. Liquid formulations, which are flavored aqueous solutions, syrups, or suspensions, are administered directly into the child's mouth by drop, spoon, or oral dispenser or incorporated into the child's food [1]. Pediatric formulations offer flexibility for dose adjustment, while at the same time remaining within the effective therapeutic range. For decades, syrups and suspensions have been considered as the favorable type of dosage form in which to administer medicines to young children [2]. The development of fixed-dose combinations formulations is becoming increasingly important from a public health perspective. This kind of formulations have advantages when there is an identifiable patient population for whom treatment with a particular combination of actives in a fixed ratio is safe and effective and when all the actives contribute to the overall therapeutic effect [3]. Liquid formulations facilitate oral administration and enhance children treatment adherence [1].

Sulfamethoxazole (SMZ) and trimethoprim (TMP) inhibit bacterial synthesis of tetrahydrofolic acid, the physiologically active form of folic acid and a necessary cofactor in the synthesis of thymidine, purines, and bacterial DNA [4]. Established indications of this combination are infections of the sinuses, ears, lungs, and urinary tract, and infections due to *Salmonella*, *Nocardia*, *Brucella*, *Stenotrophomonas maltophilia*, *Pneumocystis jirovecii*, and *Toxoplasma* [5]. Some of these conditions are treated in children [6-9]. In this group of patients, antibiotic resistance [10, 11], tolerance [12], and adverse reactions as hepatotoxicity, nephrotoxicity, bone marrow suppression, rash, electrolyte imbalance have been reported [13-15]. About 30-40% of patients on SMZ/TMP experience treatment failure [15].

In vitro dissolution tests are official test recommended by pharmacopoeias around the world to evaluate the rate and the extent of release of the drug from the dosage form over a given time. Dissolution tests were commonly carried out with the basket apparatus (USP Apparatus 1) or paddle apparatus (USP Apparatus 2). The choice of apparatus is based on the knowledge of the formulation design and the practical aspects of dosage form performance in the *in vitro* test system [16]. To date, no pharmacopeial dissolution test for SMZ/TMP oral suspensions is described. Some authors agree that it would be very helpful to use a test system that requires smaller sample sizes and smaller volumes of media but has the same reliability and predictivity as the standard test apparatus [17]. As an alternative, a mini paddle apparatus has been used for study the *in vitro* release performance of some drugs [18, 19]. About, the mini paddle apparatus might be a useful tool in characterizing drug release profiles under "standard test conditions" [17]. With this apparatus, the handling of small volumes of suspension is adequate to obtain SMZ and TMP release profiles from pediatric formulations.

Simulation of *in vivo* behavior from *in vitro* release data of some drugs has been previously described [20, 21], but to the best of our knowledge, no scientific literature is currently available on the prediction of SMZ and TMP plasma concentrations from pediatric suspensions *via* convolution approach. Simulation of drug concentrations is a powerful method to design a bioequivalent formulation during pharmaceutical development [22]. Virtual bioequivalence is a pharmaceutical concept that uses computational modelling and simulation techniques to assess the equivalence of multi-source formulations to their reference or innovator counterparts [23]. *In silico* tools can predict the bioavailability of the formulation according to the obtained dissolution profile, and it has become extremely important to ensure the safety and efficacy of oral

suspensions, especially when considering the formulation of generic drug products [16].

An *in vitro*/*in vivo* evaluation of four commercial oral suspensions containing SMZ and TMP has been reported. Dissolution and absorption profiles of both drugs were similar for all formulations however, to test *in vivo* absorption, an animal model was used [24]. On the other hand, a comparative bioavailability of SMZ in three formulations of SMZ/TMP suspensions has been published. The relative bioavailability of SMZ in two oral formulations was less than 80% [25]. Considering this background and the importance of an adequate biopharmaceutical evaluation that ensures the quality of fixed-dose combination formulations available for the child population (oral suspensions), the objective of the present work was to predict the SMZ and TMP plasma concentrations-time profiles of four commercial drug products through *in vitro* data obtained with a mini paddle apparatus, dissolution media of physiological relevance, and *in silico* methodology. The results may be important to estimate the clinical impact of SMZ and TMP of multi-source formulations available to the Mexican population.

MATERIALS AND METHODS

Reagents and chemicals

SMZ/TMP reference oral suspension (classified it as R formulation) (Bactrim® 200-40 mg/5 ml, Produtos Roche Químicos e Farmacéuticos S. A., Brasil) and three multi-source oral formulations (randomly classified them as A, B, and C formulations) were used in this study. Mexican health authorities have established Bactrim® formulation as the reference drug product [26]. HCl, sodium acetate, and phosphate monobasic and dibasic salts were acquired from J. T. Baker-Mexico (Xalostoc, Mexico). SMZ and TMP standard were acquired from Sigma-Aldrich Co. (St. Louis MO, USA).

Preliminary *in vitro* release studies

With the aim of knowing the best *in vitro* release conditions of SMZ and TMP from reference oral suspension dissolution profiles of both drugs were obtained with a mini paddle apparatus at 50, 75, and 100 rpm (Sotax AT7-Smart, Sotax AG, Switzerland) and 200 ml of 0.1 N HCl (pH 1.2) as dissolution medium. After 15 min of mechanical agitation and with the aid of a syringe, a sample of 2 ml of suspension was added to each mini vessel. Several dissolution samples were taken until 60 min and the amount of dissolved SMZ and TMP was quantified. An analytical method to identify SMZ and TMP without mutual interference has been published [27] however; both drugs were easily determined by a derivative spectrophotometric method previously developed by our research group [28].

Dissolution studies

Dissolution profiles of SMZ and TMP from oral suspensions were obtained with a mini paddle apparatus at 100 rpm. Mini vessels were filled with 200 ml of 0.1 N HCl (pH 1.2), pH 4.5 acetate buffer, and pH 6.8 phosphate buffer at 37.0±0.5 °C. Dissolution samples (n=12) were taken at 15-, 20-, 30-, 45-, and 60-min using fiberglass filters (Millipore). To quantify dissolved SMZ and TMP standard calibration curves (SMZ: 250-350 µg/ml and TMP: 10-50 µg/ml) in each dissolution medium were prepared.

Data analysis

To compare the *in vitro* release performance of SMZ and TMP (multi-source*vs.* reference) by a model-independent approach the f_2 similarity factor was calculated (similar dissolution profiles were considered if f_2 = 50-100). Furthermore, data of the percent of dissolved drug at 60 min (Q_{60}), dissolution efficiency (DE), and mean dissolution time (MDT) were calculated and statistically compared (Univariate one-way ANOVA followed by a Dunnett's multiple comparison test). DE and MDT data were calculated with the Excel add-in DDSolver program [29]. To mathematically compare the dissolution behavior of SMZ and TMP by a model-dependent approach percent of dissolved drug *vs.* time were fitted with Makoid-Banakar, Korsmeyer-Peppas, logistic, and Gompertz equation. The model with the highest adjusted determination coefficient ($R^2_{adjusted}$) and lower Akaike Information Criterion (AIC) was chosen as the best-fit model [30].

Estimation of SMZ and TMP plasma concentrations

SMZ and TMP plasma levels were estimated with *in vitro* release data from the mini paddle apparatus, a simple numerical convolution method [21, 31], and *in vivo* information of both drugs [32, 33]. Results were fitted with a non-compartment model using the Excel add-in PKSolver program [34]. Simulated peak plasma concentrations (C_{max}) and area under the concentration-time curve from zero time to infinity ($AUC_{0\text{-}inf}$) were compared with *in vivo* data by the percent of Prediction Error (%PE) that was calculated by the following equation:

$$\%PE = \left(\frac{\text{Observed parameter} - \text{Predicted parameter}}{\text{Observed parameter}}\right) \times 100 \ldots\ldots \text{Eq. 1}$$

The PE should not exceed 15% [16, 35, 36].

RESULTS AND DISCUSSION

Preliminary *in vitro* release studies

After trying different agitation rates to document the *in vitro* release of SMZ and TMP (50, 75, and 100 rpm) the best results were found with the highest agitation rate, 100 rpm (Q_{60}>80% for both drugs). Therefore, this agitation rate was chosen to carry out the final *in vitro* studies of SMZ and TMP from commercial formulations (oral suspensions).

Dissolution studies

In vitro release performance of SMZ and TMP of all used fixed-dose combination oral suspensions are depicted in fig. 1. A decrease in *in vitro* drug release proportional to decreasing acidity of the dissolution medium was observed. Considering the physicochemical characteristics of SMZ and TMP it was expected. Similar behavior of SMZ and TMP from multi-source tablets under dissolution media of physiological relevance was recently reported [37]. In the present work, to evaluate the *in vitro* release and hypothetical absorption of SMZ and TMP from pediatric suspensions, dissolution media of pH 1.2, 4.5, and 6.8 were used. The use of these conditions to test drug release through gastrointestinal tract is requested by international regulations [38]. Results of f_2 similarity factors for comparison of dissolution profiles of SMZ and TMP in dissolution media of pH 1.2 to pH 6.8 are shown in table 1. In all used conditions, dissolution curves of SMZ and TMP from multi-source oral suspensions and reference drug product were similar (f_2= 50-100) excepting for TMP of C drug product at pH 1.2 (f_2<50). Similar results for TMP from two fixed-dose combination formulation (tablets) in 0.1 N HCl (pH 1.2) as dissolution medium were found (f_2<50) [37].

Table 1: Results of f_2 similarity factor

	Sulfamethoxazole			Trimethoprim		
pH	**A**	**B**	**C**	**A**	**B**	**C**
1.2	54.91	74.36	52.02	55.72	68.66	46.69
4.5	72.64	82.67	67.49	58.29	67.58	58.37
6.8	58.63	52.45	63.46	76.74	58.96	63.27

Values of Q_{60}, DE, and MDT are shown in table 2 for SMZ data and in table 3 for TMP data. In almost all comparisons, significant differences between dissolution behavior of multi-source oral formulations and reference were found (*P<0.05). Mean values of $R^2_{adjusted}$ and AIC are shown in table 4 for SMZ data and in table 5 for TMP data. As the *in vitro* release performance of SMZ and TMP from used oral suspensions was mathematically explained by different equations, dissolution profiles comparisons by a model-

dependent approach were not possible to carried out. Similar result of model-independent and model-dependent comparisons of SMZ and TMP dissolution profiles from generic formulations (tablets) were found [37]. For SMZ and TMP in oral formulations, model-independent comparisons reflect significant differences in *in vitro* release performance.

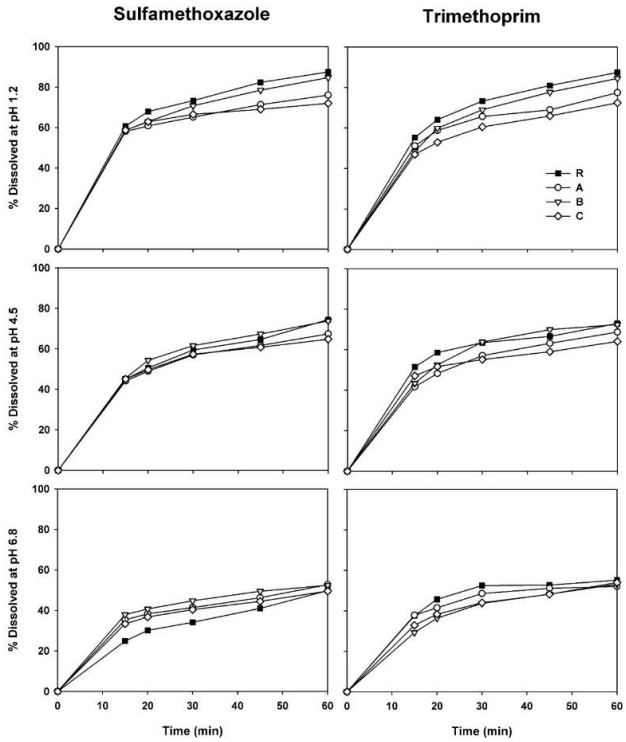

Fig. 1: Dissolution profiles of SMZ/TMP reference oral suspension (R) and multi-source oral formulations (A, B, and C). Data expressed as mean, n = 12

Table 2: Model-independent parameters of SMZ from multi-source oral formulations (A-C) and reference (R)

Parameters	Sulfamethoxazole			
	R	A	B	C
	pH 1.2			
Q_{60} (%)	87.43±0.14	75.97±0.46*	84.65±0.27*	71.93±0.20*
DE (%)	65.36±0.18	58.16±0.63*	62.56±0.27*	57.73±0.29*
MDT (min)	15.15±0.14	14.06±0.42*	15.66±0.15	11.85±0.12*
	pH 4.5			
Q_{60} (%)	74.95±0.38	67.36±0.46*	73.77±0.47	64.77±0.19*
DE (%)	51.78±0.09	49.19±0.48*	53.26±0.37*	48.97±0.08*
MDT (min)	18.54±0.21	16.19±0.22*	16.65±0.51*	14.63±0.12*
	pH 6.8			
Q_{60} (%)	49.79±0.70	52.69±0.36*	52.41±0.29*	49.57±0.12
DE (%)	31.50±0.13	37.45±0.22*	39.65±0.29*	35.89±0.17*
MDT (min)	21.94±0.67	17.33±0.36*	14.62±0.12*	16.55±0.18*

Data is given as mean±SEM; n=12. *P<0.05; Q_{60}: dissolved drug at 60 min; DE: dissolution efficiency; MDT: mean dissolution time

Table 3: Model-independent parameters of TMP from multi-source oral formulations (A-C) and reference (R)

Parameters	Trimethoprim			
	R	A	B	C
	pH 1.2			
Q_{60} (%)	87.35±0.22	77.40±0.43*	84.39±0.21*	72.35±0.13*
DE (%)	63.62±0.16	56.41±0.15*	59.87±0.26*	52.54±0.10*
MDT (min)	16.30±0.16	16.25±0.31	17.43±0.16*	16.43±0.11
	pH 4.5			
Q_{60} (%)	72.95±0.46	68.70±0.89*	72.40±0.32	64.08±0.11*
DE (%)	54.88±0.26	49.24±0.35*	53.62±0.36*	48.56±0.13*
MDT (min)	14.84±0.28	16.96±0.28*	15.56±0.28	14.53±0.12
	pH 6.8			
Q_{60} (%)	55.08±0.45	52.06±0.19*	53.14±0.38*	53.98±0.50
DE (%)	42.96±0.16	40.88±0.16*	37.22±0.23*	38.21±0.26*
MDT (min)	13.17±0.38	12.88±0.15	17.97±0.21*	17.52±0.14*

Data is given as mean±SEM; n=12. *P<0.05; Q_{60}: dissolved drug at 60 min; DE: dissolution efficiency; MDT: mean dissolution time

Table 4: Value of $R^2_{adjusted}$ and AIC of SMZ data calculated to choose the best-fit model

Parameters	Sulfamethoxazole			
	Makoid-Banakar	Korsmeyer-Peppas	Logistic	Gompertz
	pH 1.2			
R	0.9794/13.06	0.9809/11.95	0.9713/15.19	0.9662/16.06
A	0.9769/6.29	0.9604/11.010	0.9413/13.95	0.9347/14.64
B	0.9983/0.29	0.9969/2.30	0.9795/13.39	0.9715/9.17
C	0.9688/7.86	0.9636/8.53	0.9755/6.17	0.9777/5.59
	pH 4.5			
R	0.9625/16.36	0.9703/15.32	0.958/16.5343	0.9513/17.5786
A	0.9800/07.58	0.9852/07.30	0.982/08.5782	0.9781/10.5354
B	0.9549/14.74	0.9301/16.29	0.9493/16.9842	0.9524/17.3609
C	0.9793/10.30	0.9592/14.18	0.9773/11.2008	0.9818/10.0624
	pH 6.8			
R	0.9808/11.2719	0.9765/11.45	0.9646/13.29	0.9538/14.90
A	0.9865/6.0787	0.9548/11.16	0.9397/12.86	0.9302/13.93
B	0.9890/4.1673	0.9897/04.26	0.9901/03.65	0.9889/03.64
C	0.9874/5.681	0.9825/7.40	0.9771/08.19	0.9727/08.80

Data is given as mean, n=12. Reference (R) and multi-source formulations (A-C); AIC: Akaike Information Criterion

Table 5: Value of $R^2_{adjusted}$ and AIC of TMP data calculated to choose the best-fit model.

Parameters	Trimethoprim			
	Makoid-Banakar	Korsmeyer-Peppas	Logistic	Gompertz
	pH 1.2			
R	0.9822/13.70	0.9700/17.16	0.9881/11.27	0.9860/12.16
A	0.9225/19.11	0.938/17.83	0.9434/17.85	0.9437/17.90
B	0.9667/18.02	0.9505/20.90	0.9811/13.97	0.9825/12.92
C	0.9832/10.87	0.9831/11.83	0.9897/8.13	0.9890/8.39
	pH 4.5			
R	0.8933/18.25	0.9068/18.20	0.9167/17.18	0.9187/16.89
A	0.9798/10.91	0.9547/15.70	0.9750/12.08	0.9802/10.79
B	0.9855/09.09	0.8960/22.82	0.9526/18.34	0.9647/16.43
C	0.9596/09.87	0.9703/08.63	0.9675/09.03	0.9657/09.74
	pH 6.8			
R	0.8481/18.01	0.7202/21.61	0.7531/20.60	0.7686/20.06
A	0.9515/10.57	0.8674/17.23	0.8896/16.27	0.9001/15.75
B	0.9667/13.66	0.9438/17.24	0.9628/15.09	0.9719/16.63
C	0.9728/12.014	0.9755/11.39	0.9803/10.51	0.9816/10.26

Data is given as mean, n=12. Reference (R) and multi-source formulations (A-C); AIC: Akaike Information Criterion

Estimation of SMZ and TMP plasma concentrations

Using *in vitro* release data from dissolution media of physiological relevance and a mini paddle apparatus SMZ and TMP plasma concentrations were predicted as described above and results were fitted by a non-compartmental model. Estimated C_{max} and $AUC_{0\text{-}inf}$ were compared with *in vivo* data and %PE values were calculated. Data are shown in table 6.

Table 6: Value of %PE for C_{max} and $AUC_{0\text{-}inf}$ calculated to validate the simulation of plasma levels of both drugs

Parameters	Sulfamethoxazole				Trimethoprim			
	R	A	B	C	R	A	B	C
	pH 1.2							
C_{max}	4.50	17.11	8.72	21.75	-0.33	3.67	-5.22	9.91
$AUC_{0\text{-}inf}$	-1.24	11.93	3.33	16.67	8.52	18.94	11.66	24.22
	pH 4.5							
C_{max}	17.77	28.28	19.29	29.31	9.45	21.08	10.07	20.48
$AUC_{0\text{-}inf}$	13.22	24.14	14.61	25.00	23.58	33.51	24.21	32.85
	pH 6.8							
C_{max}	45.39	42.27	42.78	45.75	31.48	36.39	33.68	32.65
$AUC_{0\text{-}inf}$	42.63	38.97	39.29	42.60	42.33	46.65	44.38	43.48

%PE-Percent of prediction error, C_{max}-Peak plasma concentration, $AUC_{0\text{-}inf}$-Area under the curve from zero time to infinity

PE values<15% for C_{max} and $AUC_{0\text{-}inf}$ of SMZ and TMP were achieved for R and B drug products only at pH 1.2. In the remaining conditions PE>15% for at least one pharmacokinetic parameter was obtained. The mini paddle apparatus and dissolution media of pH 1.2 were adequate to predict the *in vivo* performance of SMZ and TMP from reference formulation. These conditions seem appropriate to evaluate the *in vitro* release performance of SMZ/TMP from multi-source oral suspensions since by obtaining PE values<15% the probability of having similar plasma levels to those generated by the reference formulation in an *in vivo* study is high. Ríos-Rodríguez *et al.*, [37] found PE<15% for C_{max} and $AUC_{0\text{-}inf}$ of SMZ and TMP of reference formulation (tablets) only at pH 1.2, which agrees with the

present work where the most acidic dissolution medium seems to be the one indicated to theoretically generate drug plasma levels similar to those observed in humans and therefore, to evaluate the *in vitro* release performance of multi-source fixed-dose oral formulations.

It is important to note that both drugs within the same formulation should generate PE values of C_{max} and $AUC_{0\text{-}inf}$ within the internationally established criteria to ensure the safe interchangeability of SMZ/TMP oral suspensions or at least to have better chances of finding bioequivalent products. Even though commercial drug products are available to the population, post-marketing surveillance is always recommended [39, 40] but in case of development of new formulations, the use of predictive *in vitro-in silico* studies to simulate the *in vivo* performance is needed, and it has gained acceptance in the regulatory decision-marketing process [16]. The use and importance of dissolution test for oral suspensions has been document for several authors [41, 42].

This is the first work that estimate SMZ and TMP *in vivo* behavior from multi-source oral suspensions using *in vitro* data generated by the mini paddle apparatus and dissolution media of physiological relevance. In the work, dissolution profiles of SMZ and TMP showed similarity in practically all the used conditions (f_2=50-100). However, the prediction of the pharmacokinetic parameters of SMZ and TMP in two multi-source formulations did not meet the established criterion which can mean negative clinical implications in the treatment of children. The dissolution conditions must be discriminative for both drugs and thus differentiate the quality of drug products with adequate *in vitro* release conditions. *In vitro-in silico* studies may be a key tool to indicate the safety and efficacy of the dosage forms and anticipate the risk of bioinequivalence [16]. As bioequivalence of two commercial SMZ/TMP oral suspensions was reported [32] it is important to carry out more research in this regard to find *in vitro-in vivo* correlation. These results provide useful information for post-marketing supervision of the commercial formulations available to the population.

CONCLUSION

The *in vitro* release data of SMZ and TMP from oral suspensions have been obtained with a mini paddle apparatus and dissolution media of physiological relevance. Dissolution profiles were compared with model-independent comparisons and significant differences were found in almost all dissolution parameters which suggests absorption differences and therefore, in the manifestation of the therapeutic effect. The mini paddle apparatus at 100 rpm and 0.1 N HCl (pH 1.2) as dissolution medium were the best conditions to predict the *in vivo* performance of reference formulation. Predicted C_{max} and $AUC_{0\text{-}inf}$ of the reference drug product and one multi-source formulation showed PE values less than 15% so the *in vitro* comparisons did not reflect the ability to predict the hypothetical *in vivo* performance of SMZ and TMP from oral suspensions. Using this approach, it was possible to make a qualitative investigation of fixed-dose combination formulations indicated for the child population. Based on the results, it can be concluded that it is necessary to carried out *in vivo* studies with the used drug products to relate *in vitro* data with *in vivo* behavior.

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Nil

AUTHORS CONTRIBUTIONS

JC Ruiz-Segura and JR Medina-López conceived and designed the experiments; JM Ríos-Rodríguez, FD Reyes-Ramírez and CE Velázquez-Sánchez performed the experiments; JM Ríos-Rodríguez contributed to the drug simulations, FD Reyes-Ramírez carried out the statistical analysis, CE Velázquez-Sánchez searched bibliographic data, JC Ruiz-Segura and JR Medina-López wrote the paper. All authors have read and agree to the published version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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