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INVITED REVIEW

The use of GA-RxODE (Genetics Algorithms and Running simulations from Ordinary Differential Equations-based model) method to optimize bioequivalence studies

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Abstract

Bioequivalence (BE) studies are prerequisite in generic products approval. Normally, they are quite simple in design and expensive in execution, and sometimes suffer ethical questioning. Genetics Algorithms and Running simulations from Ordinary Differential Equations-based model (GA-RxODE) is a multipurpose method used in pharmacokinetic (PK) optimization. It can be used to complete concentration–time (C–T) missing data. In this investigation, GA-RxODE was applied in BE field. For this purpose, three BE studies were selected as a source data comprising formulations of metformin, alprazolam and clonazepam. From them, five blood samples values per volunteer-round from specific preset times were chosen as if BE study was carried out with five instead of the classic 10–20 samples. With the five values of each volunteer a complete C–T curve was simulated by GA-RxODE and certain PK estimation parameters (as maximum concentration, C_{max}, and area under C–T curve from zero to infinite, AUC_{inf}) were elicited. Finally, with these modeled parameters, a BE analysis was performed according to certain regulatory agencies guidances. Some results, expressed as geometric mean ratios of compared formulations and their 90% confidence intervals (CI90), were as follows: Metformin C_{max} = 0.954 (0.878–1.035), AUC_{inf} = 0.949 (0.881–1.022); Alprazolam C_{max} = 1.063 (0.924–1.222), AUC_{inf} = 1.036 (0.857–1.249), Clonazepam C_{max} = 0.927 (0.831–1.034), and AUC_{inf} = 1.021 (0.931–1.119). All CI90 were inside the 0.8–1.25 BE range. In summary, the simulated data were bioequivalent and non-significantly different from original studies' data. This raises the opportunity to perform more economic BE studies to build reliable PK estimation parameters from a few samples per volunteer.

KEYWORDS
bioequivalence studies, generic drugs, genetics algorithms, NLME models, pharmacokinetic

Abbreviations: AUC_{inf}, area under curve from zero to infinity; AUC_t, AUC from zero to the determined last time; BE, bioequivalence; C_{max}, maximum concentration; cRMSE, corrected by mean root-mean-square error; CROs, Contract Research Organizations; C–T, concentration–time; GA-RxODE, genetics algorithms and running simulations from ordinary differential equations based model; HPLC, high-pressure liquid chromatography; k_a, absorption constant; k_e, elimination constant; MPE, mean percentage error; NLME, non-lineal mixed-effect model; PK, pharmacokinetic; R, reference formulation; T, test formulation; t_{max}, time to C_{max}; V_d, apparent distribution volume; λ_z, final elimination slope.

1 | INTRODUCTION

From the 1970s, regulatory agencies have placed great emphasis on pharmaceutical products bioavailability as a way to promote the manufacturing of better-quality medicines and avoid ineffectiveness–toxicity problems.[1,2] However, this emphasis has implied a great technical adjustment that progressively affected the entire production network, and such adjustment, far from has reduced costs, has triggered them to unacceptable levels.

In a context of lessen health costs, generic medication is a resource that countries could exploit. A generic drug is a formulation that contains the same active principle (in salts, isomers, or crystalline forms), the same excipients, and the same dosage strength under the same pharmaceutical form as a former brand name of product, the so-called innovator.[3] The innovator is exclusive in the pharmaceutical market until its patent expires, from that moment anyone in the industry may copy it. In such case, certain laboratory may manufacture the generic following the same procedures as the one which designed and approved the innovator.[4] Because generic drugs do not undergo all the required clinical investigation for a New Drug Approval, they should be cheaper than innovators.[5–7]

Several countries around the world have been defining the characteristics and requirements for their generic drugs, either through legal provisions from their regulatory agencies or through laws enacted by their parliaments. For instance, the American legislation, by the Hatch-Waxman Act of 1984, may authorize a given laboratory to produce and sell generic products whenever it could demonstrate that they have the same quality and are bioequivalent to innovators, and of course, do not infringe their patents.[4,8]

In Argentina, the word "generic" only indicates the WHO International Non-proprietary Name (INN) of a drug substance. And although Argentina does not have specific regulations for generic drugs like United States or the European countries, in 1999 its regulatory agency, ANMAT, established the bioequivalence (BE) standards for high sanitary risk products marketed in its territory.[9] These standards can be taken as a basis for exchangeability among the products marketed in Argentina, either if they were considered generics or not.

According to our experience, BE studies in Argentina are simple to design, expensive to execute, and complex to solve certain aspects. The BE demonstration between two pharmaceutical formulations, the generic and the innovator (in this study, also called test or T and reference or R, respectively), involves carrying out a randomized, cross-over clinical trial in healthy volunteers to determine similar drug plasma levels comparing certain pharmacokinetic (PK) estimation parameters, a fact considered as surrogate variable of similar therapeutic effect.[10,11] The more determinations carried out, the more precise an obtained PK profile is assumed to be; but this also implies more costs and, especially, more volunteers' exposure to hospital milieu, a fact that causes discomfort and increases the infection risk, and can be ethically questionable.

We have recently studied the potential of the multipurpose tool, written in R language, GA-RxODE for the PK studies.[12] GA-RxODE comprises two routines, GA (Genetics Algorithms) and Rx-ODE (Running simulations from Ordinary Differential Equations based model). The RxODE part sets the non-lineal mixed-effect (NLME) model to be followed, offering a framework for simulation criteria. The GA part obtains the best parameters that fit the simulation criteria defined by investigators, combining and selecting data matrices by Mendelian principles and the Natural Selection.[13] Because GA is a heuristic approach used in artificial intelligence to optimize available data, the entire process allows to analyze large amounts of data in the NLME context reducing restrictions can be imposed by the principle of maximum entropy, as shown in drug modeling.[14] Since there is an infinite combination of parameters that could satisfy a model for a given set of data points, it would be computationally unfeasible to perform a brute force or grid search of such parameters. Furthermore, noisy, collinear, or poor-quality datasets tend to produce singular matrices or non-converging results when conventional optimization methods are applied. In this scenario, GA is a very robust routine and guarantees to converge into a solution.

Once the investigator sets the desired model and put the source values to which it should be adjust, GA-RxODE generating a series of concentration–time (C–T) curves that progressively converge toward the preset data. For each modeled subject (e.g., animal, volunteer, patient, etc.), Rx-ODE produces initially 100 randomly simulated curves and contrasts them with a loss function L^{12} that includes the source values, so curves can be ordered from best to worst fit into 100 vectors or "genes". Then, GA proceeds cyclically; analyzes the genes, retains the best 10, recombines the next 40 among them, selects and randomly mutates 25 of the previous 50, and replaces the 25 worst vectors by new ones from Rx-ODE. Finally, the next cycle is restarted when these new 100 vectors are compared again with L and reordered for GA task. Each cycle is called a "generation" and the procedure continues for 100 cycles or until the best convergence is achieved; in addition, every 10 generations, the best vector is further optimized by the Levenberg–Marquardt algorithm.[15] The choice of 100 vectors and 100 generations is arbitrary, but these figures are large enough to give GA-RxODE a "generous" search space to achieve model convergence under all circumstances. Likewise, the random but uniform distributed mutation done in the genes should also guarantee the proposed solution will not fall outside of such space.[16] The final GA-RxODE construct, called "token- or para-data", has less experimental error, mainly intra-individual, and can be used by further procedures. Token data also enable to analyze trends like moving average and create much more robust population PK models.[12]

An issue addressed in our previous study was the construction of reliable simulated C–T curves using few determinations as if there were missing data. It was demonstrated that with fewer determinations per volunteer the initially obtained population PK model was conserved. It also established a minimum of three determinations in order to maintain the model, whenever these followed the order, baseline–maximum–minimum, simulating a peak-valley PK behavior.[12]

Therefore, if classical BE studies employ 10–20 determinations per volunteer to build C–T curves and obtain useful PK estimation parameters to claim BE, the aims of this work are as follows: to

demonstrate that GA-RxODE can simulate C–T curves but from 5 determinations per volunteer instead the usual number, and to demonstrate that these curves are also suitable to study and claim BE.

2 | MATERIALS AND METHODS

2.1 | Source data origin and trials conducting

During the first decade of the 21st century, *Química Montpellier SA*, as part of the *Bagó Group*, developed a series of studies to demonstrate BE between certain proprietary solid oral formulations (T) and the corresponding classic brand names (R) for its commercialization abroad. These studies were carried out by local independent Contract Research Organizations (CROs) in accordance with all regulations valid at that time and following appropriate ethical requirements. Even though the demonstrated BEs were not published because they were developed exclusively for regulatory purposes, their results can be employed for this research as original, source, and raw or real data indistinctly.

In brief, the BE trials were performed according to classic double-blind randomized crossover design on a minimum of 24 both genders healthy subjects.[17] The volunteers gave their written informed consent to participate in studies and underwent two doses in two rounds, one for T and one for R, separated by a wash-out period greater than five elimination $t½$ of the drug under study. According to the drug to be measured, extraction times and hospital stay were calculated. Blood samples were processed and stored at −70°C until biochemical analysis. Each completed trial produced approximately 600–700 samples that were analyzed under high-pressure liquid chromatography (HPLC) and UV light detection set at 240 nm wavelength.

2.2 | Usual obtaining procedures for PK estimation parameters

From 10 to 20 determinations from each volunteer and round, individual C–T curves were constructed. From each C–T curve, individual PK estimation parameters were obtained as follows[18]: the maximum concentration (C_{max}) and the time to reach it (t_{max}) through direct recording; the area under the curve to last recorded time (AUC_t) through Trapezoidal sum method; the final elimination slope (λ_z) and the $t½$ by last concentrations' natural log-conversion; the residual area by dividing the last concentration by λ_z, and the AUC to infinity (AUC_{inf}) by adding residual area to AUC_t.

2.3 | GA-RxODE modeling

To reproduce BE trials using GA-RxODE C–T simulation, three original studies were selected, one with the antidiabetic Metformin, and two with the benzodiazepines, Alprazolam, and Clonazepam. The three trials were primarily selected on the source data affordability, whose declassification was made 10 years after their submission for commercial registration. The drug type and its therapeutic relevance were used as secondary selection criteria.

Source data were extracted from final reports to calculate average C–T curves. Each curve included all values (T plus R) per time. Average values were log-transformed and PK parameters, t_{max}, λ_z, and $t½$ were directly calculated in order to select five preset times as if only five samples were extracted from each volunteer during a real study. As initial assumption, the sampling times were selected arbitrarily under the aforementioned peek valley concept to cover the typical PK profiles of drugs under study: one basal, one during the absorption phase, one close to C_{max}, and two during the elimination phase. Using plasma concentration values taken from the source data for each preset time, new C–T curves per volunteer-round were modeled by GA-RxODE, implemented in R programming language, following a monocompartmental model with absorption by default. Before to continue, a minimum population model for each drug was constructed to verify the accuracy in simulation. Finally, from these simulated curves, appropriate individual PK estimation parameters for T or R formulations were obtained according to the procedures described in Section 2.2.

2.4 | BE procedures

According to most regulatory agencies, the BE proof is achieved when the geometric mean ratio between T and R—or point estimate—of C_{max}, AUC_t, and AUC_{inf} approaches to one and its 90% confidence interval (CI90) lies inside the 0.8–1.25 range.[18] With these PK estimation parameters from each drug's formulations, the CROs analyzed whether the data were bioequivalent using specific software of that time (PKCalc® and WinNonLin 4.0®) and prepared the final reports on BE following US Food and Drug Administration (FDA), European Medicines Agency (EMA), and Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT) Argentina recommendations.[9,19,20]

With this methodology, it was attempted to demonstrate BE with T and R formulations' parameters derived from simulated data. Thus, values for C_{max}, AUC_t, and AUC_{inf} were log-transformed and processed to acquire and test the point estimates. By mixed-effect two-way ANOVA[21] (or BE ANOVA), the sources of variances could be discriminated and elicit the proper residuals to build the CI90s. To verify that each CI90 fit within the proposed range, a two one-sided (TOS) test was used.[22] To construct individual PK estimation parameters after C–T modeling in R language and perform the BE analysis as described, an Excel 2013 for Windows® sheet was specifically programmed.

2.5 | Statistics

Most C–T curves were drawn using natural log-conversion of concentration values in order to rectify the elimination slopes, except those that represented population models. Because of PK estimation parameters values have a log-normal distribution, their natural log-conversion was absolutely necessary before to initiate any

analysis. So, data were expressed as median (range), mean (SD), mean (CI95), or geometric mean (CI90) where necessary. The deviation between actual and simulated values of PK estimation parameters were evaluated via mean percentage error (MPE) and corrected by mean root-mean-square error (cRMSE). Overall data were analyzed by a fixed-effect two-way ANOVA[23] and the differences between point estimates were studied by mean difference test linked to this ANOVA. For the statistical analysis and graphic rendering, another Excel 2013 for Windows® sheet was prepared.

3 | RESULTS

This investigation has generated, processed, and confronted a huge data amount. For instance, the development of the simulated PK estimation parameters of one drug under study requires at least 10 000 matrices among data extrapolation, residuals analysis, and covariate comparisons. This is the main reason because three and no more studies were included as source data; and the second reason is only those three trials were declassified at first instance. Furthermore, it should be considering the studied drugs are widely used and recognized worldwide; Metformin is first-line drug for diabetes and metabolic syndrome treatments, and the two benzodiazepines are among the most prescribed anxiolytic drugs.

Tables 1 and 2 present some characteristics of source BE trials. In all studies, there were a prevalence of male volunteers. The number of determinations per volunteer-round used to build individual C–T curves were 18 in the metformin study and 11 in the 2 benzodiazepine studies.

Figure 1 displays the redrawn average C–T curves of each studied drug. At first glimpse, Metformin and Alprazolam appear to follow a monocompartmental model, while Clonazepam displays a clear bicompartmental model. Furthermore, Figure 1 indicates the PK parameters necessary to select the five times. Considering t_{max} and λ_z-derived $t½$, the better representative times to perform simulations were, for Metformin: 0, 1, 3, 6, and 12 h, respectively; for Alprazolam: 0, 0.5, 1, 8, and 24 h, respectively, and for Clonazepam: 0, 1, 2, 12, and 48 h, respectively.

Using a monocompartmental model with absorption (as surrogate of non-compartmental PK) and the five times, the simulated model parameters shown in Table 3 were obtained. Figure 2 complements such information displaying the population models calculated with these parameters. As seen, the five points were enough to generate a representative and conserved model (left) whose average curves-CI95s contains almost all means of the real data (right). If a mean lies inside CI95 of another mean, this is indicative they are non-significantly different and no more evidence are needed. Likewise, Figure 3 further contribute to shows similarity between simulated and original data. Here, T and R values from the two sources for C_{max} and AUC_{inf} are illustrated individually per volunteer (markers) and by the representative geometric mean-CI90 (line-bar). In addition, it includes MPE, cRMSE, and p (from the ANOVA analysis) values. Visually, the data distribution appears to be similar between the simulated and real ones, but the deviation between original and simulated data implies a rough variation of −20% to 26% (MPE) and 8%–57% (cRMSE), with the highest percentage variation originated by Clonazepam AUC. The two-way ANOVA indicates there were non-significant differences. All of this indicate that GA-RxODE simulated curves would exhibit a similar behavior to the original ones.

Returning to the second aim, Table 4 shows the non-compartmental PK estimation parameters needed to analyze BE, but derived from the GA-RxODE-simulated curves. And Table 5 shows the results of classic BE analysis achieved with simulated PK parameters, compared with the BE data provided by source trials. This table is organized around the point estimates of each necessary PK parameter: C_{max}, AUC_t, and AUC_{inf} for the studied drugs, and their CI90s. All CI90s of simulated data are within the 0.80–1.25 range (second column) and the performed TOS tests were significant ($p < .05$; values

TABLE 1 General characteristics of original BE studies

	Metformin	Alprazolam	Clonazepam
Year of performing	2004	2007	2008
Brand-name Test formulation	DBI®	Tranquinal®	Neuryl®
Brand-name Reference formulation	Glucophage®	Xanax®	Rivotril®
Pharmaceutical form	Coated tablets normal release	Tablets	Tablets
Single dose used (mg) per volunteer-round	500	0.5	1

TABLE 2 Demographic characteristics of original BE studies

	Metformin	Alprazolam	Clonazepam
n volunteers completed the trial	24	24	24
Age (years)	32.5 (8.9)	31.8 (10.4)	32.1 (8.5)
Sex (% Female)	25	34	29
Height (m)	1.68 (0.09)	1.68 (0.09)	1.72 (0.07)
Weight (kg)	69.6 (11.1)	65.9 (11.5)	72.9 (6.3)
Adverse events during or after trial execution	Not registered	Not registered	Not registered

Data expressed as mean (SD) except sex (%).

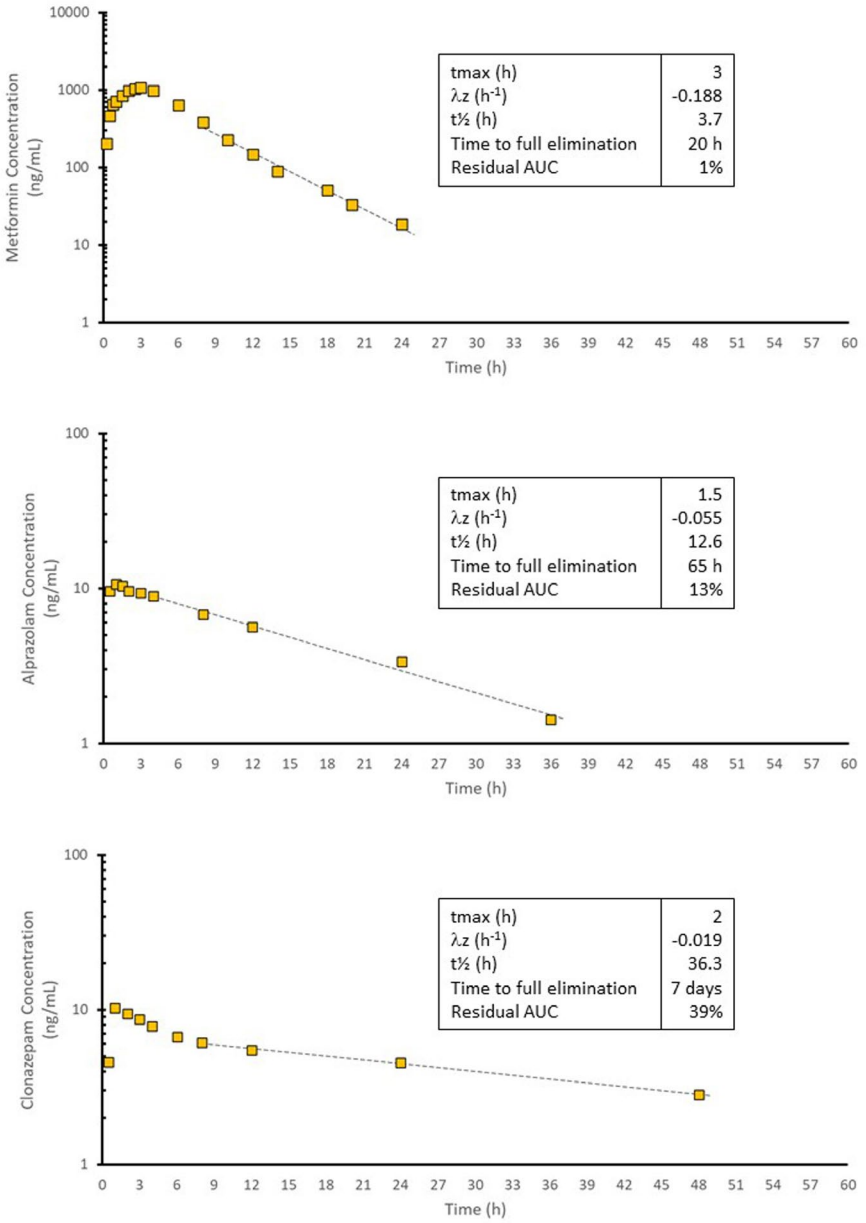

FIGURE 1 Average C–T curves for the drugs under study. Each C–T point comprises the mean of all T and R formulations values, so $n = 48$. The data were extracted from original BE studies, redrawn (using log-transformation) and recalculated to produce PK parameters necessary to estimate the possible sampling times according to each drug (see the inset)

not shown). The simulated values are close to the original data (central column). However, in the original data series, all CI90s are also inside the 0.80–1.25 range, except the CI90 of Alprazolam AUC_{inf} that touch the upper limit and so, its TOS test was non-significant. Finally, the log-transformed mean differences between simulated and original point estimates for all PK parameters were also non-significant (last two columns). Thus, BE can be demonstrated using C–T curves modeled by GA-RxODE from a few data points.

4 | DISCUSSION

Nowadays, the societies increasingly demand from the pharmaceutical industry, directly or indirectly, products of the highest quality and inexpensive. A way to elicit them is through generic drugs production that would operate as more accessible therapeutic alternatives that can be exchanged without further ado at the patient-consumer request.[24] However, the term generic medication arouses in a good portion of the population a certain, but perhaps unjustified, skepticism and concern.[7,25,26]

To be exchangeable, a generic should be equal to its innovator in almost all aspects. However, nearly its entire essence is based on the BE demonstration and obviously, neither its manufacturing process nor its proven BE assure its quality in terms of therapeutic effectiveness and safety.[26] The manufacturing of generic products should lower the health costs, but both Good Manufacturing Practices and Good Clinical Practices applied today actually rises the productive costs,[27] making this approach less profitable. Therefore, companies could cut expenses and the final quality of a given generic medication might suffer.

On the other hand, BE trials are carried out in healthy volunteers from whom blood is drawn in clinical setting to safeguard any contingency. Beyond the financial compensation they receive, each volunteer

TABLE 3 Monocompartmental model estimate parameters after GA-RxODE simulation

Drug	Metformin		Alprazolam		Clonazepam	
Formulation	T	R	T	R	T	R
k_a (h^{-1})	4.76 (0.48)	4.77 (0.98)	9.99 (3.70)	4.52 (1.54)	0.71 (0.29)	0.95 (0.46)
k_e (h^{-1})	−0.243 (0.064)	−0.234 (0.056)	−0.061 (0.030)	−0.057 (0.046)	−0.024 (0.027)	−0.037 (0.029)
V_d (L)	520.60 (140.20)	505.90 (106.40)	94.40 (19.75)	99.86 (20.50)	270.31 (47.20)	232.05 (92.35)

Data expressed as mean (SD).

Abbreviations: k_a, absorption constant; k_e, elimination constant; V_d, apparent distribution volume.

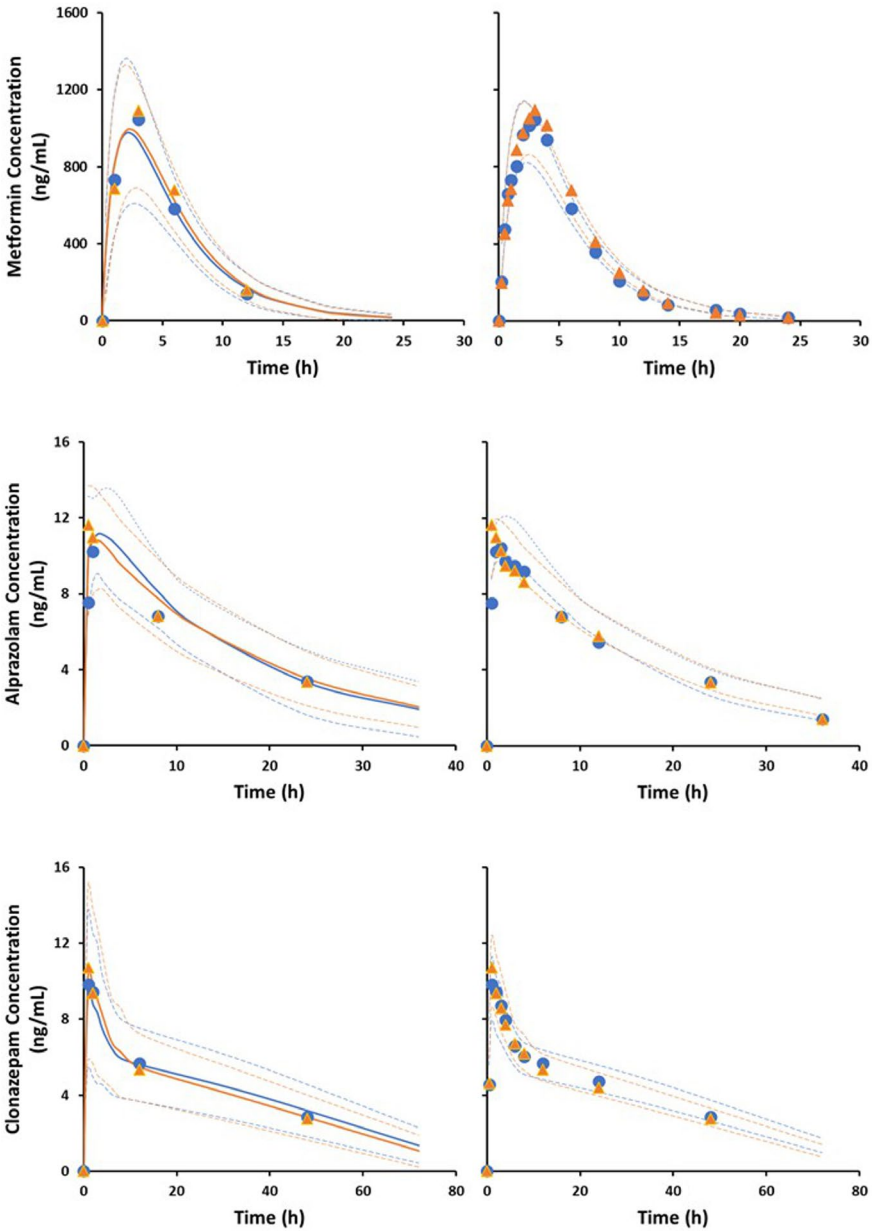

FIGURE 2 PK population models for T and R formulations of the drugs under study built using parameters derived from five sample points (data extracted from original BE studies). For comprehensive purposes, the graphic was divided into two parts; on the left, the model is represented as average C–T curve ±standard deviations and, on the right, the model is represented as CI95 of the mean. Likewise, on the left, the five determinations that produced the model and, on the right, all average original C–T determinations (11 or 18 by drug) are superimposed in order to indicate that model represents all of values. Circles T (test) data, n = 24; triangles R (reference) data, n = 24

loses about 200–300 ml of blood per BE study and is exposed to the hospital environment for 24–36 h with possible risk infection. So, why someone healthy should be treated as an ill person can revive classic controversies in bioethics.[28,29]

All these circumstances should prompt regulatory agencies to look for new, original ideas and points of view to satisfy the balance among quality, economy, and benefits of the products that must authorize. In this prospect, GA-RxODE could be extremely useful. This multipurpose procedure was used in this investigation to construct PK profiles with only five C–T values in order to demonstrate BE between generic-innovator formulations, as if a BE study was made with few determinations per volunteer-round.

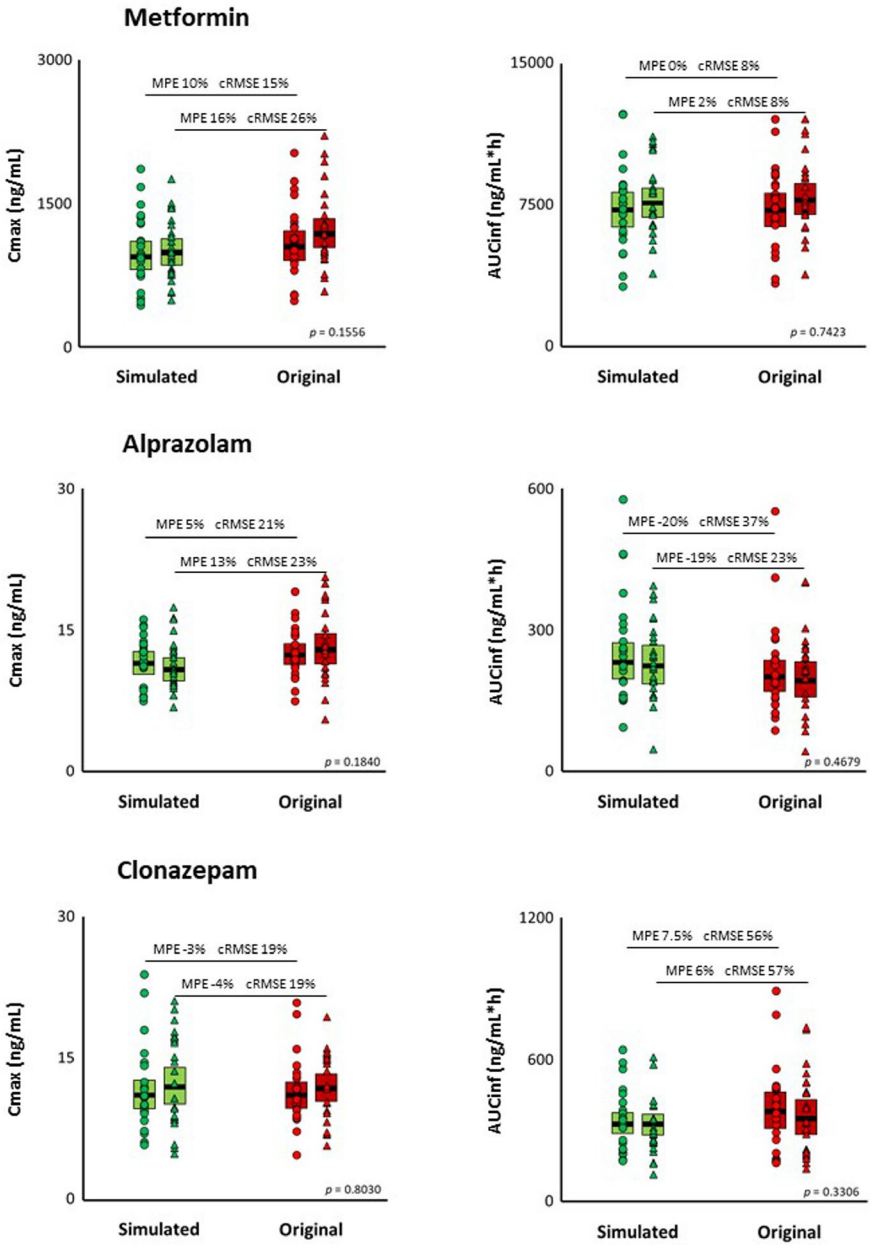

FIGURE 3 Comparison between simulated and original relevant PK estimation Parameters, C_{max} and AUC_{inf}, from the studied drugs. In all cases, markers represent individual non-log-transformed volunteer's value per formulation, and horizontal lines and bars represent the geometric mean and CI90, respectively, of each data group. The figure includes the deviation estimation of simulated over real data (MPE and cRMSE) and the statistical analysis (fixed-effect two-way ANOVA of log-transformed data). There were no statistical differences between simulated and real parameters. Circles T (test) data, $n = 24$; triangles R (reference) data, $n = 24$

In regard to above, this research found: First and foremost, GA-RxODE was capable of building a series of simulated PK curves with similar characteristics to real ones, using less data points. Second, these C–T curves were useful to obtain reliable PK parameters to estimate BE between formulations. Third, these simulated BE parameters did not show to be significantly different from the original BE data provided by the three studies. For the studied drugs, the simulated results would be similar to the source BE data. This would imply the modeling for BE generated by GA-RxODE is efficient.

However, three issues are needed to be discussed before continuing, the BE studies in Argentina, the arbitrary choice of five determinations to do simulations instead of other number, and the great source of variation in Clonazepam AUC between simulated and real data.

As mentioned before, in Argentina there are no regulatory provisions on generic medication; therefore, any product whose patent has expired can be copied without any other requirement if it is already marketed in the country. However, BE studies are used in this country to evaluate the interchangeability of products whose active ingredients are contained in extended release oral pharmaceutical forms or have high health risk.[9] The last included, those indicated for severe pathologies such as epilepsy (Antiepileptics) or HIV infection (Antiretrovirals), those that present a great number of pharmacovigilance reports (Clozapine), or those that have a complicated PK (Clopidogrel). In the past, the costs to perform BE studies were low enough to allow making them for export. But today the situation has changed dramatically and the domestic costs are very high, especially the analytical procedures and the devices maintenance, so the studies are expensive and limited to regulatory requirements. In this regard, the Argentinean regulatory agency, ANMAT, in some cases shows certain ethical-legal objections toward these studies and takes its

TABLE 4 Non-compartmental PK estimation parameters employed in BE analysis derived from the modeled C–T curves taking 11 determinations (n = 24 volunteers per formulation drug)

Drug	Metformin		Alprazolam		Clonazepam	
Formulation	T	R	T	R	T	R
C_{max} (ng/ml)[a]	1006.3 (374.6)	1038.2 (336.4)	11.87 (2.49)	11.41 (2.81)	11.71 (4.62)	12.88 (4.96)
t_{max} (h)[b]	2.5 (2.0–4.0)	2.5 (0.5–6.0)	0.5 (0.5–4.0)	0.5 (0.5–4.0)	2.5 (2.0–4.0)	2.5 (0.5–6.0)
λ_z (h^{-1})[a]	−0.23 (0.07)	−0.24 (0.06)	−0.06 (0.03)	−0.05 (0.02)	−0.03 (0.01)	−0.03 (0.01)
$t½$ (h)[b]	2.80 (1.96–5.95)	2.81 (2.12–5.17)	12.81 (5.63–44.50)	13.15 (5.55–29.10)	22.40 (16.19–86.29)	19.93 (11.72–87.77)
AUC_t (ng/ml × h)[c]	7134.3 (6376.9–7981.6)	7545.1 (6908.1–8240.7)	188.56 (170.99–207.83)	182.83 (159.50–209.57)	234.61 (211.84–259.84)	232.88 (209.14–259.31)
AUC_{inf} (ng/ml × h)[c]	7238.4 (6473.8–8093.3)	7628.8 (6983.6–8333.6)	231.91 (200.20–268.63)	223.78 (189.87–263.75)	330.83 (297.97–367.33)	324.15 (290.64–361.52)

Data expressed as: [a]mean (SD); [b]median (range); and [c]geometric mean (CI90).

Abbreviations: AUC_{inf}, area under curve from zero time to infinite; AUC_t, AUC from zero to the determined last time (Metformin 24 h, Alprazolam 36 h, and Clonazepam 72 h); C_{max}, maximum concentration; R, reference formulation; T, test formulation; $t½$, elimination half-life; t_{max}, time to C_{max}; λ_z, final elimination slope.

time in issuing and authorizing their carry out. Faced with these difficulties, we have thought to apply GA-RxODE in the BE field.

Since a PK model generated by GA-RxODE could be produced with up to three determinations and even so preserve its internal congruency,[12] five values seemed a better option because they could give more clarity to the model avoiding greater external dispersion, as shown in this work. The key concept here is to provide the model with a peak-valley behavior, so any erratic and underfitted curve that may appear will be discarded by GA routine if does not fulfil the required profile. For this reason, it was preferred the sampling distribution described in Material and Methods to cover the curve development. And although there might be excellent specific software to calculate the best time values for a given C–T curve, in this investigation, they were chosen "by hand" knowing t_{max} and $t½$ of drugs to be studied. To end, a simple proof that the points chosen were appropriate is the closeness between the λ_z values shown in Figure 1 and the k_e values shown in Table 3 (middle row).

As seen in Figure 3, there were great internal dispersion in Clonazepam AUC_{inf} values detected by cRMSE test, between simulated and original data. Its exact significance is unclear. Thinking about it and reviewing the situation, we believed that, unfortunately, the original Clonazepam study had a design error that apparently did not influence the BE demonstration in the past, but the procedure followed here to mend the situation could be the cause of such dispersion. The design error was revealed during this investigation when, initially, residual AUC was recalculated and yielded a value of 39%, when the usual is 20% or less. This would indicate that an additional determination should have been included after 48 h. Therefore, the simulated Clonazepam curve was prolonged until 72 h and AUC72 was used instead of AUC48, since what was to be compared in the figure was its derived AUC_{inf}. Under this procedure, the residual AUC was reduced to 28%, but it would not seem to be capable to decrease the internal dispersion or perhaps, it could even add more. This is an open issue and additional research would be required to be solved.

In sum, the world interest in generic products is rising, proof of this is the increase in the number of papers about "BE studies and generic medication" that have been published in the last 50 years, from 1 in 1973 to 80–90 in the last year.[30] In this context, this investigation intended to show the capabilities of GA-RxODE in BE.

So, whether it is applied to BE studies, these trials would be cheaper and safer inasmuch as they will require only 30%–50% of the usual determinations and a shorter hospitalization time. This opens the possibility that GA-RxODE model simulation might be used as a reliable substitute to the traditional methodology in BE studies, and even could be used during the planning or design phase to simulate possible scenarios and optimize the protocol preparation.

This investigation is a first step in this area and covering solely the comparison between conducted trials and their derived simulated data, there are some limitations: the conclusions cannot be generalized to high variability PK drugs or those with multiple and erratic metabolism, until more BE trials are available to us.

We are now able to extend the GA-RxODE capabilities in this field planning new BE comparisons with older studies, including

TABLE 5 Bioequivalent point estimates between T and R formulations for the simulated and original data

Parameter[a]	Simulated BE[b]	CV% intra[c]	CV% inter[d]	Original BE[b]	CV% intra[c]	CV% inter[d]	Difference[e]	p[f]
Metformin								
C_{max}	0.954 (0.878–1.035)	17	31	0.885 (0.822–0.954)	16	31	−0.085	.1251
AUC_{t24}	0.946 (0.877–1.019)	16	25	0.914 (0.857–0.975)	14	25	−0.073	.1111
AUC_{inf}	0.949 (0.881–1.022)	16	25	0.926 (0.867–0.989)	14	25	−0.064	.1394
Alprazolam								
C_{max}	1.046 (0.926–1.181)	26	10	0.960 (0.836–1.103)	29	8	0.002	.5149
AUC_{t36}	1.031 (0.877–1.213)	34	8	1.043 (0.896–1.213)	32	19	0.036	.6905
AUC_{inf}	1.036 (0.857–1.249)	40	22	1.043 (0.861–1.253)*	40	23	0.036	.6906
Clonazepam								
C_{max}	0.927 (0.831–1.034)	23	31	0.934 (0.853–1.023)	19	25	−0.072	.1625
AUC_{t48}	1.007 (0.920–1.103)	19	24	1.043 (0.958–1.128)	17	27	0.014	.5873
AUC_{inf}	1.021 (0.931–1.119)	19	24	1.078 (0.934–1.243)	30	43	0.052	.7170

Abbreviations: As Table 2; CV% inter, CV interindividual; CV% intra, CV intraindividual.

[a]All simulated and real parameters exhibited BE; TOS test $p < .05$ except (*) ns.

[b]Data expressed as geometric mean T/R ratios and their CI90s.

[c]CV% intra, values obtained from residual MS of BE ANOVA.

[d]CV% inter, values obtained from volunteer MS minus residual MS of BE ANOVA.

[e]Difference between simulated BE and original BE point estimates; each value expresses the log-transformed mean difference (T-R simulated minus T-R original).

[f]Mean difference test using residual MS from fixed-effects two-way ANOVA.

those non-bioequivalents, and awakening the general interest of this tool in such BE trials. If potential users, that is, academic environment, pharmaceutical industry, health authorities, ethical committees, and others, consider this approach useful and enable its development, a series of new kind of BE studies can be designed and carried out.

DISCLOSURE

HAS is Química Montpellier employee.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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