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https://doi.org/10.3395/2317-269X.00814

Registration of generic topical dermatologic medications: Brazilian scenario and studies to demonstrate bioequivalence

Registro de medicamentos genéricos tópicos dermatológicos: cenário brasileiro e estudos para demonstração de bioequivalência

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Received: Jul 3, 2016
Approved: Nov 9, 2016

ABSTRACT

Comparing the number of approvals granted for topical drug products by the FDA and by Anvisa, as well as the number of tests required by these and other international agencies at the time of registration, it becomes clear that the increased flexibility of the Brazilian regulatory requirements has resulted in a larger number of topical medicines on the market, without a guarantee of bioequivalence between the different formulations considered generic. For this reason, the aim of this study is to discuss, from the point of view of Brazilian researchers, the methodologies that could possibly be used in Brazil for the reasons mentioned above, the most urgent being a revaluation of Brazilian legislation concerning bioequivalence of these products. Among the approaches considered are: in vitro release test, in vitro permeation, pharmacodynamic test (only for corticoids), and dermatopharcokinetic and dermal microdialysis. We conclude that, firstly, based on the simplicity of the methods, as well as the ease for their implementation, parameters for the in vitro approach must be defined. Later, a wider discussion involving Anvisa, the scientific community and the industrial sector should be sought, aiming to assess the technical and economic viability of the adaptation to the Brazilian scenario in relation to the use of the in vivo methods discussed here.

KEYWORDS: Medicine Topical; Bioequivalence; Registration of Medicines; Agência Nacional de Vigilância Sanitária; Sanitary Surveillance

RESUMO

Comparando-se o número de registros concedidos para produtos tópicos pelo *Food and Drug Administration* (FDA) e pela Agência Nacional de Vigilância Sanitária (Anvisa) com o número de testes exigidos por essas e outras agências internacionais no momento do registro desses medicamentos, fica claro que a flexibilização das exigências regulatórias brasileiras vem proporcionando um maior número de medicamentos tópicos no mercado, sem que haja garantia da bioequivalência entre as diferentes formulações consideradas produtos genéricos. Diante disto, o objetivo deste trabalho é discutir, sob o ponto de vista do pesquisador brasileiro, as metodologias possíveis de serem utilizadas no Brasil para esta finalidade, considerando ser premente uma rediscussão da legislação brasileira no que concerne a bioequivalência destes produtos. Dentre as metodologias abordadas estão estudos de liberação e permeação *in vitro*, ensaio farmacodinâmico de branqueamento (exclusivo para os corticoides), dermatofarmacocinética e microdiálise dérmica. Concluímos que, inicialmente, baseados na simplicidade dos métodos, bem como na facilidade de implementação, parâmetros para a abordagem *in vitro* devem ser definidos. Posteriormente, uma discussão ampla envolvendo Anvisa, comunidade científica e segmento industrial deveria ser buscada, visando avaliar a viabilidade técnica e econômica de adequação à realidade brasileira, no emprego dos métodos *in vivo*, aqui discutidos.

PALAVRAS-CHAVE: Medicamento Tópico; Bioequivalência; Registro de Medicamento; Agência Nacional de Vigilância Sanitária; Vigilância Sanitária

INTRODUCTION

The Bioequivalence (BE) assessment of topical products has been much debated, especially by the Food and Drug Administration (FDA), since the 1998 publication of a draft guide to BE studies on topical products using the methodology of dermatopharmacokinetics (DPK)[1]. This guideline stopped being recommended in 2002 mainly because of issues about the suitability and reproducibility of the method regarding the analysis of topical preparations based on tretinoin[2,3,4,5]. Ever since then, several international workshops have been held to discuss this procedure. Among the proposed challenges, we can mention the evaluation of the concentration of the drug in dead tissues; understanding the relationship between drug concentration in the stratum corneum (SC) and topical efficacy; the reproducibility of the DPK method and the ability thereof to reliably differentiate different formulations[6]. Except for topical products with corticosteroids, for which the use of the pharmacodynamic approach is accepted by the FDA, clinical trials are the only route for the approval of a generic topical drug. Therefore, the DPK techniques and the *in vivo* cutaneous microdialysis are in focus and guide different approaches, considering the evaluation of the active ingredient in the different layers of the skin. Of these, only DPK is accepted for the evaluation of topical products in countries like South Africa and Japan. In the same context, it is worth noting the importance of the *in vitro* release and permeation studies as an attempt to evaluate succinct modifications in the formulations, and/or to envision possible correlations between *in vitro* and *in vivo* procedures. In Brazil, comparing the number of registrations granted by the FDA and the National Health Surveillance Agency (Anvisa) regarding topical dermatological formulations with the number of tests required by these and other international agencies at the time of registration, we can conclude that more flexible regulations at the national level provide for more generic and similar generic dermatological drugs that are interchangeable in the Brazilian market, without there being any effective guarantee in terms of BE among their different formulations[7]. In the international literature, it is well established that, for dermatological products, after topical application, the concentration of drug found in the biological fluid may not be related to therapeutic efficacy. As a result, the use of methodologies to assess the BE of these products continues to be a major challenge[6]. Based on the above, the objective of this study is to discuss the possible methodologies to be used in Brazil for this purpose, considering that reviewing the Brazilian legislation on the BE of topical products is urgent.

METHODS FOR ASSESSING THE BIOAVAILABILITY/ BIOEQUIVALENCE OF TOPICAL PRODUCTS

The main methods to be considered when determining topical Bioavailability/Bioequivalence (BA/BE) can be divided into two approaches: (1) *in vitro*, including release and cutaneous permeation trials, and (2) *in vivo*, (e.g. vasoconstriction), DPK and dermal microdialysis (MD), as shown in Figure 1.

RELEASE AND PERMEATION TRIALS IN VITRO

In vitro approaches are usually performed through Franz diffusion cells (FDC) (Figure 1). We propose using this method for evaluating the release kinetics of the active component(s) of each formulation. Since its introduction, FDCs have been used in a number of studies, including those for assessing the absorption of local action formulations, transdermal patches, cosmetics, skin care products and pesticides[8]. In a few words, these devices consist of two compartments, a donor and a receptor, separated by a membrane (of cellulose, silicone etc.). The receiving compartment is filled with a solution favoring *sink* conditions, i.e., conditions far from the saturation limit of the dynamic system[9]. This enables a continuous and unidirectional flow of the drug. In the schematic diagram of a vertical FDC, we can see a "jacket" through which the temperature-controlled water is recirculated so we can perform the tests at the desired temperature. The passage of the active ingredient through the membrane is monitored by periodic sampling of the receptor solution from the collecting duct, and is subsequently analyzed through suitable analytical techniques. The inability of the Release Study (RS) to correlate with the *in vivo* results is mainly due to the impossibility of obtaining data representing the interaction between the formulation (including the fillers) and the skin, in particular, with SC[1]. However, while the *in vitro* release test itself is not a substitute for BA/BE tests, the FDA guide states that a manufacturer can make minor changes to their product and use the *in vitro* release test to demonstrate the "similarity" between them. Thus, in the case of Brazilian legislation, it is possible to foresee a greater use of *in vitro* release trials (similar to the dissolution profile for solid forms of oral use), as part of a series of tests that can be used to establish the therapeutic equivalence of topically applied medicinal products.

In the evaluation of the *in vitro* permeation, the procedure is similar to the RS, with changes in the membrane and in the duration of the study. Ideally, we should use human skin to evaluate the permeation properties of a drug. However, samples of adequate size and quality to perform the experiments are not sufficient and of difficult access to most researchers[10]. A wide variety of animal skin models are suggested as substitutes for human skin, and these have been used to evaluate the permeation of different drugs[11,12,13,14]. Such models include rodents, primates and pigs. With regard to porcine skin, some *in vitro* studies have reported that SC thickness and biophysical parameters (diffusivity and water permeability coefficient) are correlated with those of human skin [*in vivo*] (Table)[15]. In view of this, porcine skin, mainly of the ear, has been used rather often. It is worth noting that skin from the dorsal and ventral parts of the animal's body has also been used, making harvest and treatment procedures (including dermatomization) faster and leading to lower variability in the experimental data. These different skin substrates must be stored at -20°C and therefore, prior to use, the integrity of these substrates must be assessed[1].

COMPARATIVE CLINICAL TRIALS

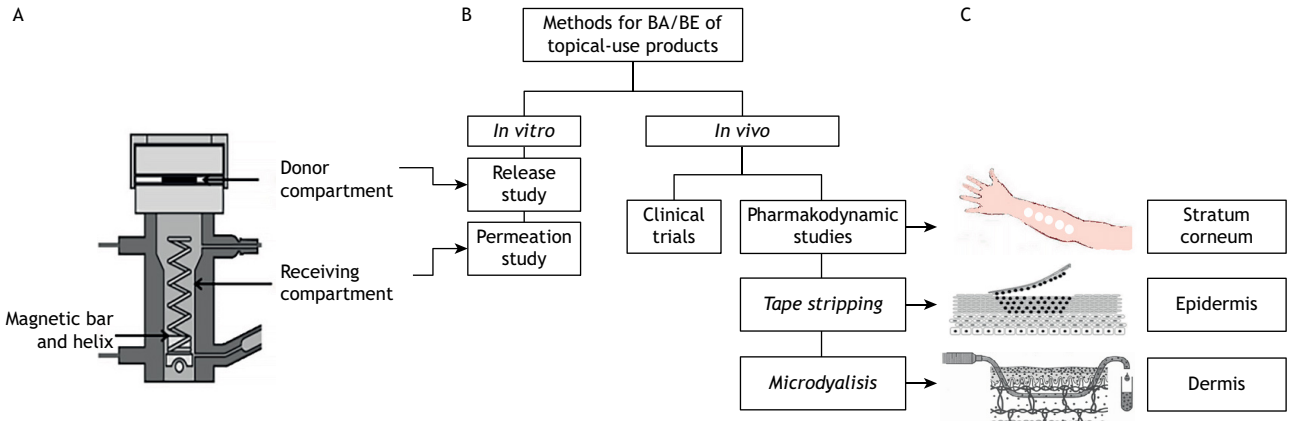
Comparative clinical trials are considered the gold standard for the evaluation of the BE of any drug. However, for topically applied drugs, these tests are generally not very sensitive due to high interindividual variability. They are usually time-consuming, expensive and require a large number of participants[16]. These conditions often make them impossible, especially in developing countries like Brazil. Therefore, the need for tests that can safely replace them should be fulfilled. From the bioethical point of view, the decrease in the use of human beings in clinical trials is a goal that mobilizes researchers around the world. The substitution of an *in vivo* method by an alternative *in vitro* solution or other *in vivo* tests using a smaller number of volunteers has great scientific and social relevance.

PHARMACODYNAMIC TEST

As previously mentioned, the only FDA-accepted methods for evaluating the BE of topical products are limited to clinical and pharmacodynamic trials. The pharmacodynamic test (bleaching test) is easy to perform. Participants in the research are exposed to small amounts of formulation for a short period of time. The method is relatively reproducible and requires a small sample when compared to comparative clinical studies (12 participants for the pilot study and 40-60 for the main study). However, such studies are only applicable for corticosteroid drugs[17]. The pharmacodynamic response of glucocorticoids is associated with their ability to produce vasoconstriction in the microvasculature of the skin, leading to bleaching at the application site (Figure 1C). Therefore, the bleaching intensity can be correlated with the power, as well as with the degree of drug release in the SC. Several studies have shown the correlation between these results and those of clinical efficacy in patients[18,19]. At the same time, some authors have also shown that it is a saturable test, that is, after a certain level of product applied, the test could not detect differences of low intensity among the formulations. Despite this limitation, this method is accepted by the FDA for the evaluation of generic topical drugs. According to the FDA's Guide to *Topical Dermatologic Corticosteroids: In vivo Bioequivalence*[20], carrying out two tests is imperative: a pilot (preliminary), whose purpose is to establish the relationship between dose duration and pharmacological response of the reference product (Figure 1C), and another major and extended trial in which the *in vivo* responses of the test product and reference product are compared *in vivo* using appropriate statistical tools. A critical factor to be considered in this experiment is the volume of preparation applied, which becomes even more complex for preparations in which the drug is presented in different pharmaceutical forms (e.g. solutions and creams). Considering the Brazilian legislation, this would not be a factor of relevance, since the drugs to be considered generic need to have the same pharmaceutical form. For the accomplishment of the said study, the chromometer is the equipment accepted by the FDA, regardless of all the points indicated[21].

DERMATOPHARMACOKYNETIC EVALUATION

DPK is a methodology in which the amount of drug found in the SC is quantified based on the post-application and post-removal of the tested product, using the *tape-stripping*[22] methodology.



FDA: *Food and Drug Administration*; BD/BE: Bioavailability/Bioequivalence

Source: Adapted from Hanson Research[62](A), Wiedersberg et al., 2006[47](B) and Adapted from Au et al.[61](C).

Figure 1. (A) Schematic model of the Franz cell; (B) Main methods for the determination of the BE of topical-use products (the italic methods are still under evaluation by the FDA; (C) representative figures of the bleaching test, DPK and MD.

Table. Comparison of biophysical parameters of porcine and human skin *in vivo*.

Skin	H[1] (μm)	D[2] ($cm^2 \times s^{-1}$)	K_p[3] ($cm \times h^{-1}$)
Porcine ear (*in vitro*)	11.8 ± 4.0	3.2 ± 1.5	5.8 ± 1.1
Human (*in vivo*)	10.9 ± 3.5	3.0 ± 1.5	6.1 ± 1.4

Source: Sekkat et al. (2002) (apud Tabosa[8]).
H[1]: SC thickness; D[2]: Diffusivity of water through the skin; $_KP3$: Coefficient of permeability of water through the skin

By evaluating transepidermal water loss (TEWL) and weighing the tapes before and after the tape-stripping procedure, one can evaluate the drug permeation profile in the SC versus the relative depth (Figure 1C)[23,24,25]. The main assumption of this technique is that the amount of drug recovered from the SC, the main barrier of percutaneous absorption, is directly related to the amount of active principle that reaches the target cells. Thus, this methodology can be used instead of clinical trials in BE studies of topical products, or more specifically for products with SC action. According to the original protocol published by the FDA, an approach to DPK can be made by analyzing the drug in at least eight places: four different sites exposed to the product are collected at successive intervals, corresponding to absorption kinetics, whereas other four (or more) are collected at different sites, as well as exposed to the drug as described above. These represent the elimination kinetics[1]. Next, we build a chart (amount on the skin vs. time) and evaluate the parameters: maximum absorption time (T_{max}), maximum concentration (C_{max}), area under the curve of the total study time (ASC_{0-t}), half-life ($T_{1/2}$) and elimination constant (K_{el}) of the drug (FDA, 1998)[1], as we can see in Figure 2A.

A few years later, the following improvements to this method were proposed: (1) better cleaning of the excess formulation at each application site at the end of the absorption period; (2) inclusion and determination of drug concentration in the first two tapes; (3) increase in the number of tapes collected combined with a method that guarantees the removal of almost all the SC; (4) better control of the sampling area with the tapes, which should be within the area of application of the drug (to avoid edge effects); and (5) an analysis procedure that allows the determination of the drug in all the tapes, alone or associated in groups[22]. This new data analysis, together with a differentiated protocol, in which the absorption and the elimination are evaluated in only two different points, demonstrated that the comparison of the BE between different formulations can be carried out more easily and become more reproducible, since the analyses are done in duplicate, as shown in Figure 2B[26]. In order to ensure that practically the entire SC was removed, the evaluation of transepidermal water loss is an essential parameter. Thus, it is recommended that the final SC collection should occur when the TEWL value reaches ~ 120 g/m²h. By evaluating 0.75% metronidazole gel formulations following the improvements in the above-mentioned DPK technique, our team was able to show differences between the formulations tested (with high reproducibility and low variation in the data) when analyses of drug concentration in the SC were made either by area or volume, both for the time of absorption and elimination, considering or not the amount of SC collected, corroborating the studies done by N'stempfer[22,27]. However, new studies using drugs with different physicochemical characteristics should be performed.

In fact, the DPK procedure relies on a relatively inexpensive technique that requires a small number of individuals. According to studies carried out with formulations containing econazole[28] and corroborated by our research group[27], in the evaluation of formulations containing metronidazole, the use of 14 healthy volunteers was enough to safely demonstrate the BE of topical products.

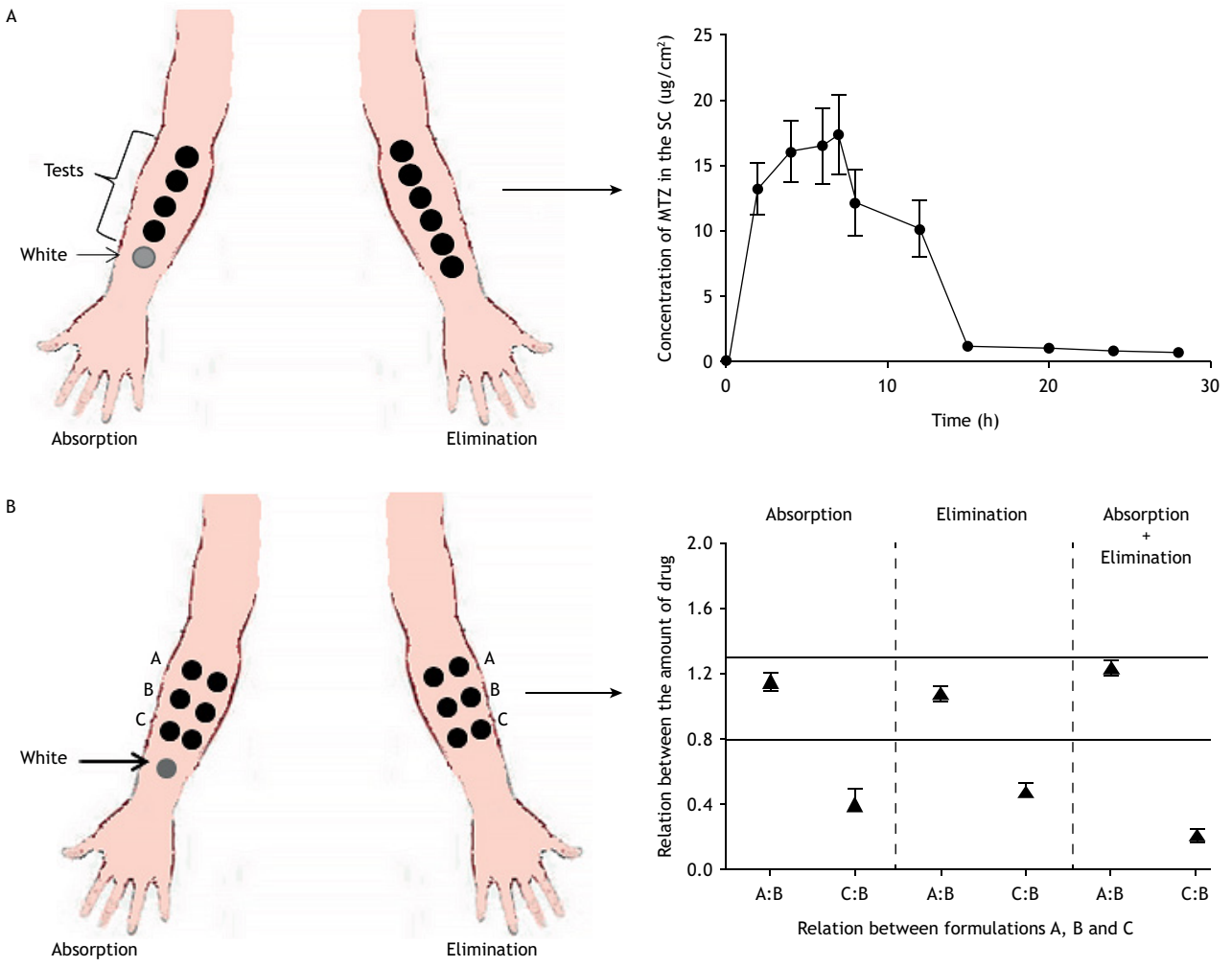
The DPK evaluation has also been performed *in vitro* using porcine skin. However, the skin elimination mechanisms in this condition are not fully functioning and the lipophilic nature of some drugs, such as econazole[28], may hinder the DPK analysis within the usual *in vivo* elimination time. It worth noticing that *in vitro* DPK studies, in porcine dorsal skin, have shown low variability of experimental data (Figure 3)[27]. At the same time, some *in vitro*/*in vivo* correlation studies using this data have been described in the literature[2,29,30,31,32,33].

DERMAL MICRODIALYSIS

Among the other techniques mentioned, MD is an *in vivo* methodology that aims to assess the concentration of free drug in the extracellular fluid in tissues or organs[34]. Since there is no loss of body fluids, it can be used in small animals and in humans, through different types of probes in different areas[35,36,37]. In the case of MD, this technique enables the quantification of the drug content in the extracellular fluid of the dermis/epidermis. Moreover, samples can be continuously collected over a given period. In fact, many researchers have reported evaluating the penetration of drugs from topical formulations using MD studies in mice, pigs and humans[38].

The recommended probe in this case is of a linear type. It must be inserted between the epidermis and the dermis (Figure 1C). The depth of insertion of the probe is a critical step and will influence the results, considering that, according to Fick's law, the thickness of the tissue to be permeated is inversely proportional to the flow of the drug. The probe serves as an artificial vessel, allowing the exchange of small diffusible molecules from the extracellular fluid to the probe and vice versa. Thus, similar to oral absorption experiments, this method can provide concentration vs. time profiles, allowing pharmacokinetic measurements. Another point is that test and reference formulations can be tested simultaneously on each volunteer from various sampling points. This is of paramount importance as it reduces interindividual variability, thus reducing the total number of volunteers required to establish the BE of topical medicinal products[39]. An unfavorable point is that microdialysis may not be suitable for all types of analytes, like large molecules and lipophilic molecules, which may be more challenging samples[40].

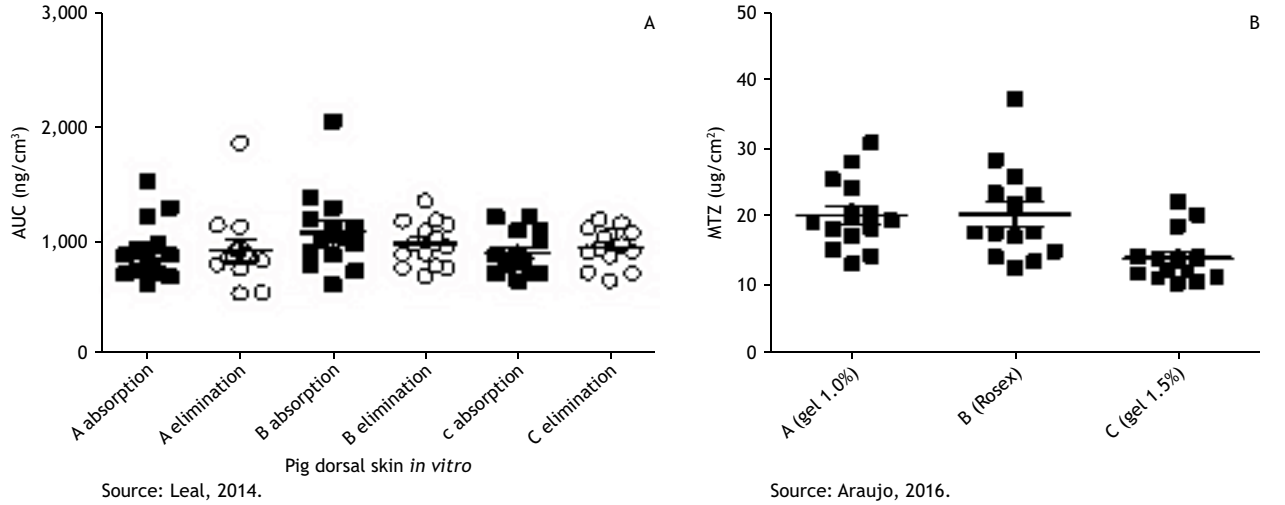
Microdialysis is a more invasive procedure when compared to the DPK procedure and even if there are different types of probes, there is a common deployment difficulty in all of them, as well as in the ability to determine the amount of molecules to be recovered. This, in turn, is influenced by the flow, by the characteristic of the perfusion liquid, by the type of molecule and surface area of the membrane. It is noteworthy that, for each test, the *in vivo* recovery of the probe should be evaluated to allow for the correction of the analyzed samples[41,42].



MTZ: metronidazole 0,75%; EC: stratum corneum

Source: Araujo, 2014.

Figure 2. (A) DPK profile in humans of 0.75% metronidazole (MTZ) in the SC after the topical application of Rozex®® according to the FDA Guide (N = 8); (B) Evaluation of BE of 0.75% MTZ gels (test products A and test product C) compared to the reference product (product B) measured in duplicate in 14 healthy volunteers assuming an absorption time and an elimination time.



Source: Leal, 2014.

Source: Araujo, 2016.

Figure 3. (A) Drug amounts per volume unit/skin area of the pig's back for three econazole products measured after 6 hours of absorption and 17 hours of elimination (n = 14) and (B) three MTZ products measured after 6 hours of absorption (n = 14).

DETERMINATION OF BIOLOGICAL SAMPLES FROM DPK AND MD PROCEDURES

There are several reports in literature about the determination of drug concentration in the SC through tape analysis. However, most of the methods have not been described as validated according to an official guide. Some of these describe only recovery, selectivity and linearity trials[43,44,45,46,47,48,49,50,51,52,53,54,55]. The evaluation of the reliability of the data generated by the analysis of drugs in complex matrices of biological origin and corresponding to kinetic processes, i.e., concentration variation over time (MD) or depth in the SC (DPK), requires an evaluation from the point of view of bioanalysis.

In the validation of the bioanalytical method, the evaluation of some parameters should be done differently from those concerning analytical methods. In a recent update, Anvisa separated the validation guide for bioanalytical methods[56] from the guide for analytical methods[57] with which it was previously associated.

Among these parameters, the linearity evaluation is performed by monitoring the accuracy and precision at all levels of the calibration curve by the concentration calculated through the regression and not only through the determination of the coefficient of determination (r^2). This becomes important considering, in particular, that the amounts of analyte measured in each tape obtained from the drug permeation process in the SC reach different concentrations, as can be seen in the chart (Figure 2A).

In this sense, we need to evaluate the accuracy and precision of the method by performing quality control samples in at least four different levels: lower limit of quantification (LLQ) and low quality controls (LQC), medium quality controls (MQC) and high quality controls (HQC) in at least three different analytical sequences, of which at least one is different from the others. This procedure is essential since MD and DPK studies usually generate a large number of samples (*high throughput*), implying in several different analytical sequences.

At the same time, the drugs are extracted from a biological matrix (human and/or animal skin) with high possibility of interference from endogenous compounds, especially in high performance liquid chromatography with ultraviolet detection (HPLC-UV). From this point of view, the evaluation of skin component interference of at least four different research participants/animals should be done, not only the formulation without the active principle (placebo) as recommended for analytical methods. The recovery assessment by the extraction process shall be evaluated in at least three different concentration levels (LQC, MQC and HQC) to verify the homogeneity of the recovery efficiency in the calibration interval of the method. The use of a compound as an internal standard should be considered in cases of variation of the recovery process.

The stability of the drug in the skin, in extractive solution, in the tape as well as after the extraction procedure should be evaluated, as kinetic processes with several collection points and several replicates generate analytical sequences with many samples, which means long periods in the sample holder of the device equipped with an automatic sampler.

In this context, and considering that both DPK and MD methods are being recognized as promising approaches to the evaluation of BE of topical products, the development and validation of an appropriate bioanalytical methodology for *in vivo* and *in vitro* applications should be explored, considering RE n. 899/2003[57] and RDC n. 27/2012[56] from Anvisa and based on the guidance issued by the FDA in 2013 for the validation of these methods[58].

EVALUATION OF GENERIC MEDICINES IN BRAZIL

In Brazil, the current legislation for the registration of generic and similar topical non-systemic drugs does not require the submission of BE studies or clinical studies for generic applicants, as described in RDC n. 60 of October 10, 2014[58]. Thus, currently, the product may be exempt from relative BA/BE study if the test medicine has the same drug in the same concentration as the reference drug (pharmaceutical equivalents) and fillers with the same function as the comparison drug. The fillers used in the test formulation should be well established for the pharmaceutical form, type of administration and at concentrations that are appropriate to the intended function. Therefore, only the pharmaceutical equivalence evaluation (Eqfar) should be presented. However, these studies of Eqfar only evaluate the physical-chemical and microbiological parameters established in official compendia such as the Brazilian Pharmacopoeia[7]. In April 2016, with the publication of Resolution RDC n. 73, of April 7[59], no study was requested to compare the semi-solid formulations before registration and in cases of post-registration alterations. Thus, there is currently no official Brazilian guide recommending and/or orienting the manufacturers with regard to the technical-scientific bases involved in the methodologies for the execution of the test(s) or delimiting the necessary specifications. Taking into account that drug penetration into the SC is a complex process and depends, among other factors, on the physical and chemical properties of the drug, the type of formulation and association established with the formulation, we can expect that the clinical efficacy of a topical dermatological product strongly depends on the final medical product[7].

CHALLENGES IN THE CONTEXT OF BRAZILIAN LEGISLATION

Considering all of the facts above, Figure 4 describes the items that in our view need to be initially discussed and then standardized, based on the Organization for Economic Cooperation and Development (OECD) Guide 428[60] as well as on the Guidance for Industry of the FDA[2], in order to adapt them to the Brazilian reality. This standardization follows an order of priority and ease of implementation, starting with *in vitro* studies. Therefore, once the necessary modifications are implemented when registering/modifying a topical product with Anvisa, the next step should be the discussion of *in vivo* tests. In this context, the evaluation of corticosteroid products by bleaching tests should be reported separately, considering the specificity of this group of drugs. For the others, considering the

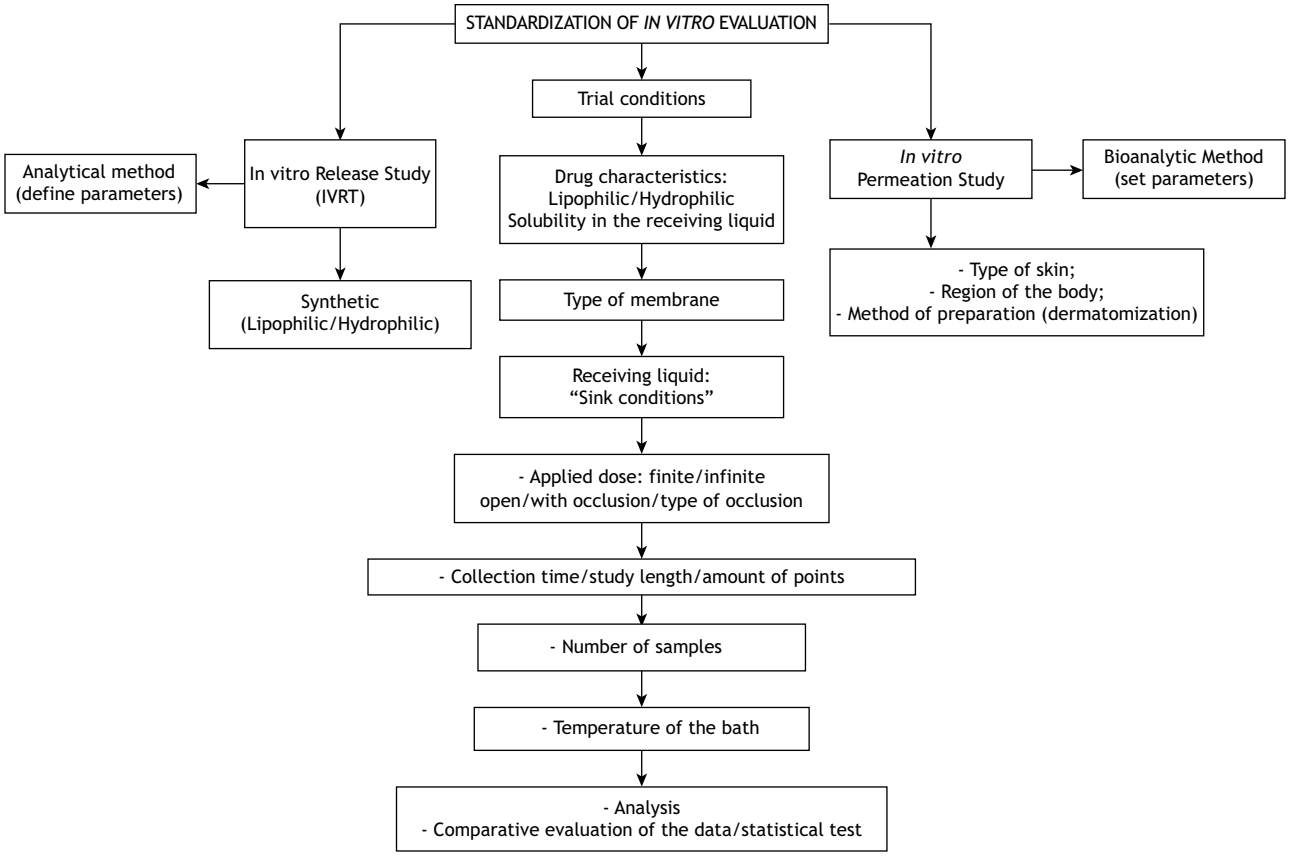


Figure 4. Workflow to be followed/evaluated in the accomplishment of BE tests of topical products and of local action in Brazil.

place of action of the drug on the skin, the methodologies to be applied should be dermatopharmacokinetics (DPK) and/or cutaneous microdialysis (MC). It is worth noting that the Brazilian pharmaceutical manufacturers, after developing the generic market, have built up the technical-scientific capacity not only to discuss possible modifications, but also to adapt to them. At the same time, there are several centers in Brazil, certified by Anvisa, that perform BE tests for oral medications, including evaluation of release kinetics as well as pharmacokinetic data, which, with their experience and competence, will be fundamental in this process. These centers can contribute to the performance of the proposed tests, due to the range of high selectivity and sensitivity analytical equipment (CLAE coupled to sequential mass spectrometry), quality assurance systems and technically qualified people already available to provide data with the necessary reliability.

CONCLUSION

These early reflections lead us to envision that, based on the relative simplicity of the *in vitro* methods as well as on their ease of implementation, parameters for the essentially *in vitro* approach should be defined initially. A broad discussion involving Anvisa, the scientific community and the industry should ensue in order to evaluate the technical and economic feasibility of its adaptation to the Brazilian reality, the use of the *in vivo* methods discussed here and to determine the BE of topical and local action products. This shall be done not only to improve the safety and efficacy control of these generic products in the Brazilian market, but also to implement analysis tools for the development of innovative products that can be more competitive in the world arena.

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Conflict of Interest

The authors report that there is no potential conflict of interest with peers and institutions nor political or financial conflicts in this study.