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The local and systemic NSAIDs evaluation using in vitro biorelevant methodologies

PhD THESIS SUMARY

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Cuprins

The bioavailability and bioequivalence assessment of topical semisolid dosage forms has been intensely debated and remains one of the challenging fields to regulate. After local application on biological barriers, permeation and penetration processes are strongly influenced by the qualitative and quantitative composition, the morphological and functional integrity of the skin or mucous membranes, the applied dose and the frequency of administration, and/or by the subsequent complex transformations. Unlike other routes of administration, each excipient in the composition may influence the amount of medicinal substance exposed to the therapeutic target, the duration of exposure, and the nature and intensity of the effect. Safety and efficacy evaluations do not have a "one size fits all" approach. Yacobi A et al (2014) noted that approaches adapted to the specificities of the pathology, the biological barrier, the medicinal substance, and the dosage forms are necessary.

Except for topical solutions, the vast majority of dosage forms intended for local administration at the level of the skin and mucous membranes are considered complex. The complexity of gels, creams, and ointments is described by the multitude of factors influencing in vivo behavior and, implicitly, by the diversity of ways in which variations of these factors can cause therapeutic failure. The risk assessment strategies for non-equivalence between multisource products are described by the FDA in specific guidelines, and the European authority recommends developing protocols by selecting applicable recommendations from a general guide (EMA, 2018). The American concept is of totality of evidence or aggregate weight of evidence. Currently, it is considered that a single method (in vitro or in vivo) cannot demonstrate the equivalence of multisource products. EMA has proposed the use of a similar concept of extended pharmaceutical equivalence. The concept document published in 2014 describes the assumed limitations, but also the potential utility of in vitro methods, clearly specifying that in the case of local administration, pharmaceutical equivalence does not guarantee bioequivalence.

In this context, the development and optimization of topical semisolid dosage forms become much more challenging compared to those specific to oral solid dosage forms. Comparative studies with clinical objectives remain an important option for regulatory authorities, despite their disadvantages and limitations (including sample size, duration, involved costs, etc.). In 1997, the FDA adopted a guide for evaluating post-approval variations specific to topical semisolid products (Nonsterile Semisolid Dosage Forms. Scale-up and Post Approval Changes. Chemistry, Manufacturing and Controls. In vitro Release

Testing and In Vivo Bioequivalence Documentation (SUPAC-SS); FDA, 1997). This document, which can be seen as a natural continuation of similar guidelines applicable to immediate and modified-release oral solid forms (FDA, 1995a,b), represents the first official use of in vitro release tests for topical semisolids. Variations were classified into three levels, depending on their potential influence on in vivo behavior. For Level 2 SUPAC-SS, which includes changes that may have a significant impact on quality and performance, comparisons of in vitro release rates between the authorized formulation, which becomes the internal reference, and that resulting from the implementation of the variation are required.

The research theme aimed to apply biorelevant in vitro methodologies in comparing multisource topical semisolid products containing ibuprofen, as a typical representative of the nonsteroidal anti-inflammatory class. The selection of this type of formulation was based on the following arguments: i) the therapeutic index of the active substance, namely the reduced risk of systemic adverse reactions after local administration; ii) the reduced complexity of the dosage form (ibuprofen being completely dissolved in the semisolid matrix); iii) the availability of a wide range of concentrations for authorized products (50-100 mg/g); iv) the major impact of the concentration gradient on penetration and permeation, and the discriminatory character of IVRT against this factor (validation criterion). The studies conducted were based on regulatory guidelines issued in the last 5 years, which will significantly influence the research and development of generic products, their availability, and therapy costs. This paper includes in vitro methodologies whose results were interpreted in relation to known advantages and limitations. In vitro release tests were developed and validated using several types of vertical diffusion cells. These were subsequently applied for comparative analysis of multisource products with known compositional differences (non-Q1). The experimental data were correlated with rheological microstructural analyses, contributing to the current efforts to validate the principles of the Topical Drugs Classification System within the Center for Drug Sciences. Elucidating the microstructure - in vitro release relationship is essential for simplifying authorization procedures and the facilitated application of the biowaiver concept adapted to topical semisolids.

The section of the doctoral thesis that presents personal contributions includes three chapters.

Chapter 4. Development and validation of an in vitro release method for ibuprofen from hydrogels containing 100mg/g ibuprofen. Application of the method in the assessment of extended pharmaceutical equivalence.

The purpose of this first set of evaluations, the results of which are presented next, published in the journal Pharmacy (Viziteu HM et al, 2024a), was to develop an IVRT methodology validated according to current guidelines and subsequently applied in comparing two topical semisolid hydrogel products containing ibuprofen at a concentration of 100mg/g (10%). The characteristics of alternative formulations, difficulties observed in demonstrating discriminatory character, and specifics of the in vitro release process, alongside potential relevance for in vivo performance, were described.

The product considered as the internal reference (comparator, according to EMA, 2018; subsequently coded R) was represented by a drug authorized at the European level, having a carbomer as the matrix former of an unidentified grade. The test formulation (coded T) was generated based on a cellulose derivative, in a research-development process by a partner of the Center for Drug Sciences.

The results confirmed the dependence of ibuprofen solubility on the concentration of absolute ethanol and led to the selection of the 50% level for the receptor medium. The complexity of the validation process was also discussed, especially in the case of formulations where low viscosity generates high release rates. The requirements to demonstrate the specificity of the IVRT method must be correlated with the procedure and the acceptance criteria for similarity, especially if the variation in active substance concentration is limited by solubility in the semisolid matrix.

Comparing multisource products and the kinetic differences observed cannot be interpreted as therapeutic non-equivalence. It represents an indicator that encompasses the influence of several factors, including the size of industrial batches, manufacturing process parameters, transformations occurred over the shelf life, the time interval between manufacturing and testing, etc. The dominant factor for ibuprofen release is the high concentration gradient, along with the reduced complexity of the hydrogel compositions, may reduce the impact of observed differences in vitro. Formulations were declared non-similar from the perspective of the extended pharmaceutical equivalence concept adopted by the European Medicines Agency.

Chapter 5. Adapting the in vitro release method of ibuprofen to the characteristics of hydrogels containing 50mg/g ibuprofen. Application of an alternative kinetic model and estimating relevance for in vivo performance.

For the development and validation of an IVRT method, certain operational parameters are preset, such as the amount of formulation dosed in the donor compartment in vertical

diffusion cell devices (when applying semisolid formulations through dosing rings with well-defined internal space, thickness, and diameter).

What is not considered is the role of all excipients present in the composition of a topical formulation. Ensuring complete dissolution while maintaining a concentration of the therapeutic entity very close to maximum solubility is one of the ways to increase thermodynamic activity. Large amounts of solubilizing agents can decrease the viscosity of the semisolid vehicle and consequently reduce diffusion resistance, generating increased release rates. If the previous chapter evaluated the in vitro performance of hydrogels containing 100 mg/g ibuprofen, the current chapter presents experimental data obtained by adapting the IVRT methodology to the characteristics of topical semisolid formulations with a concentration of 50 mg/g. The results were published in the article titled *The role of microstructural testing in the assessment of the in vitro release profiles from topical semisolid formulations*, Farmacia Journal, 2024 (Viziteu HM et al, 2024b). Reducing the concentration gradient between the two specific compartments of the vertical diffusion cells can amplify discrimination against compositional variables and can more sensitively signal suboptimal in vivo performance in comparing multisource products. The experimental plan was designed with the primary objective of analyzing in vitro release profiles and correlating differences between formulations with the kinetic model and parameters characterizing the release of ibuprofen from the semisolid matrix, in the context of possible extrapolations to local and systemic exposure consequences.

An adapted IVRT methodology was applied to a group of six topical semisolid hydrogel products containing ibuprofen 50 mg/g, authorized in the European Union. Similar to the formulations analyzed in the previous chapter, ibuprofen was completely dissolved in a matrix generated by hydrophilic macromolecular agents: hydroxyethyl cellulose (for products coded A, B, and C), carbomer (for products coded D and E), and poloxamer (block copolymer of polyethylene and polypropylene, for the product coded F). The previously developed in vitro release method was adapted by modifying the receptor medium. A phosphate buffer system pH=7.4 was selected, in which the solubility of ibuprofen allowed the maintenance of sink conditions. The aqueous solution was prepared according to USP (2023) recommendations and was degassed through vacuum filtration using artificial membranes made from cellulose ester blends (declared average pore diameter, 0.22 μm).

IVRT tests results demonstrated adequate discriminatory character against compositional variables. The reduced consistency of hydrogels containing cellulose derivative induced rapid releases, evolving to complete depletion of the donor compartment

in the use of vertical diffusion cells. Adapters for semisolids allowed dosing of larger amounts of formulation, preventing complete diffusion of ibuprofen. In the case of carbomer-based products, a reverse diffusion process was demonstrated, which did not lead to changes in the kinetic model of in vitro release.

Chapter 6. Microstructural Analysis and Evaluation of the In Vitro Performance Similarity of Ibuprofen-Containing Hydrogels

The last chapter of this paper integrates rheological parameters into a detailed assessment of similarity based exclusively on in vitro methodologies. Individual and mean diffusion profiles were compared using parametric and nonparametric tests recommended by current guidelines. The interpretation of the results, partially published (Viziteu HM et al, 2024b), was performed by correlating with available information regarding composition and microstructural characteristics, as well as with literature data related to local and systemic exposure.

Microstructural characterization of hydrogels containing 50 mg/g and 100 mg/g ibuprofen was performed using two categories of rheological analyses, oscillatory and rotational.

The analysis of the similarity of in vitro release profiles was performed by comparing two types of specific parameters, the diffusion rate and the cumulative amount recovered at the receptor compartment, calculated over different time intervals. Depending on the type of formulation (implicitly, the type of molecular agent and correlated flow properties), the release kinetics were adequately described by the Higuchi model (square root law) or, in cases of advanced depletion, by an alternative model (adaptation of the one applied in the case of the appearance of a transient boundary layer). Regardless of the experimental points (time intervals) considered, the release kinetics, experimental devices used, statistical analysis, and criteria for accepting similarity applied, the results confirmed the relationship between composition, microstructure, and in vitro performance.

The personal contributions to this doctoral thesis are highlighted in the following paragraphs, with references to the chapters where they can be found.

In the first phase, an in vitro release test (IVRT) methodology was developed, tailored to the characteristics of topical semisolid formulations (Chapter 4, paragraphs 18-26). Based on the requirements for maintaining sink conditions and using an inert support interface, a hydroalcoholic medium and an artificial membrane were selected (Chapter 4, paragraphs 18,20). Experimental conditions and operational parameters were adapted to assess a high

rate of release, leading to advanced or even complete depletion of the applied dose (Chapter 4, paragraphs 22,28).

The validation stage included demonstrating the discriminatory nature against dose and microstructure (Chapter 4, paragraphs 23,25). The results illustrated the complexity of the process of selecting the composition of alternative series (maintaining the aggregation state of the active entity while ensuring Q1/Q2 similarity, the minor impact of microstructure on in vitro performance, the major role of the semisolid matrix-forming agent (Chapter 4, paragraph 25). Statistical analyses and similarity acceptance criteria were adopted based on the comparison of the in vitro release rate and the cumulative amount released, both parameters calculated using the Higuchi diffusion model. Even under conditions of high release rate and advanced depletion of the donor compartment (containing a pseudo-infinite dose), the methodology showed adequate discriminatory character, confirming non-equivalence of Q1/Q2/Q3 for two multisource products (Chapter 4, paragraphs 27,28).

In the second chapter dedicated to personal contributions, the previously developed and validated IVRT methodology was adapted to hydrogels containing 50mg/g ibuprofen (Chapter 5, paragraphs 7-12). Two types of experimental devices were used, vertical diffusion cells and adapters for semisolids, mounted within continuous flow cells, both described in the United States Pharmacopeia, with known constructive and hydrodynamic differences. The protocol was applied to a group of non-Q1 similar multisource products (containing different macromolecular agents, neutralizers, and solubilizers) and included two types of complementary tests useful for pharmaceutical equivalence analysis: evaluating residues as the final stage of product metamorphosis through exposure to controlled, physiological temperatures, and determining the evaporation profile of volatile components (Chapter 5, paragraphs 6,13).

Significant kinetic differences were observed between the two experimental models, mainly determined by the volume of the receptor compartment and viscosity-dependent diffusion of the semisolid formulation (Chapter 5, paragraphs 15,16). The consistency of hydrogels based on hydroxyethyl cellulose, minimal among the analyzed product group, led to rapid and complete in vitro releases, especially in the first half of the testing interval, particularly when using vertical diffusion cells. Deviations from the Higuchi diffusion model and a decrease over time in discriminatory character were noted (Chapter 5, paragraph 18). This phenomenon was avoided by using adapters for semisolids, which allow dosing larger amounts of hydrogel. For formulations containing carbomer, the occurrence of reverse diffusion was suspected, without affecting the kinetic release model of ibuprofen. A

rheological test demonstrated that, at the end of the test, the donor content exhibited microstructural changes, particularly reductions in internal interactions and the flow stress (Chapter 5, paragraphs 24,25,31).

For characterizing the entire in vitro release profile, even under conditions of advanced depletion, an alternative approach to data processing was applied, representing the transposition of the transient boundary layer model (specific to lipophilic ointments containing suspended particles). It was hypothesized that rapid release leads to a continuous decrease in the ibuprofen concentration in the donor compartment, which does not behave like a pseudo-infinite reservoir. Thus, the linear dependence of the cumulative amount recovered at the receptor compartment and the logarithmic values of the sample collection time was explained. The phenomenon was considered relevant also for in vivo administration, through the depletion and continuous transformation of the semisolid layer, with the distribution of the active substance at the local and/or systemic level (Chapter 6, paragraphs 13,36).

The sources of in vitro performance differences demonstrated were identified through a set of rheological analyses, rotational and oscillatory, useful in identifying the microstructural characteristics recommended by regulatory authorities (Chapter 6, paragraphs 15-19). As a secondary physicochemical parameter, water activity was determined, another indicator of the complex interactions generating the specific behavior of semisolid matrices (Chapter 6, paragraphs 20,24). Linear viscoelastic regions, tensions and areas (points) of flow, areas under the curve, and viscosity values estimated at three shearing speeds within thixotropy tests, creep curves, and structural recovery profiles were analyzed.

The results, interpreted in the last chapter of personal contributions, confirmed a marked dependence of the rheological behavior on the nature of the macromolecular agent. The work proposed a data analysis model capable of identifying kinetic changes occurring during IVRT tests. Specific parameters for comparing multisource products were estimated over different time intervals and later included in the similarity analysis (Chapter 6, paragraphs 37-41). The complex relationship between composition, microstructure, and diffusion in release was confirmed and represents one of the central elements of the Topical Drug Classification System. The results obtained argue for relaxing the strict requirements for Q1, Q2, and Q3 similarity, applicable in the bio-waiver procedures specific to topical semisolid pharmaceutical forms, and advocate for the use of comparative in vitro methodologies as an alternative in estimating local and systemic exposure.

Bibliography

Bodenlenz M, Yeoh T, Berstein G, Mathew S, Shah J, Banfield C, Hollingshead B, Steyn SJ, Osgood SM, Beaumont K, Kainz S, Holeček C, Trausinger G, Raml R, Birngruber T. Comparative Study of Dermal Pharmacokinetics Between Topical Drugs Using Open Flow Microperfusion in a Pig Model. Pharm Res. 2024 Feb;41(2):223-234. doi: 10.1007/s11095-023-03645-3.

Effendy I, Kwangsukstith C, Lee JY, Maibach HI. Functional changes in human stratum corneum induced by topical glycolic acid: comparison with all-trans retinoic acid. Acta Derm Venereol. 1995 Nov;75(6):455-8. doi: 10.2340/0001555575455458.

EMA, 2018. European Medicine Agency (EMA). Committee for Medicinal Products for Human use (CHMP). Draft guideline on quality and equivalence of topical products, 2018. London (UK). https://www.ema.europa.eu/documents/scientific-guideline/draft-guideline-quality-equivalence-topical-products_en.pdf

FDA, 1997. US-Food and Drug Administration. Guidance for industry. Nonsterile semisolid dosage forms. Scale-up and post approval changes. Chemistry, manufacturing and controls. In vitro release testing and in vivo bioequivalence documentation (SUPAC-SS), 1997. Bethesda (MD). Available at: https://www.fda.gov/media/71141/download.

FDA, 2012. US-Food and Drug Administration (US-FDA. Center for Drug Evaluation and Research (CDER), Office of Regulatory Policy. Draft guidance on acyclovir 5% ointment.

FDA, 2022a. US-Food and Drug Administration (US-FDA). Center for Drug Evaluation and Research (CDER), Office of Regulatory Policy. Draft guidance on In Vitro Permeation Test Studies for Topical Drug Products Submitted in ANDAs. October 2022. Available at: https://www.fda.gov/media/162475/download.

FDA, 2022b. US-Food and Drug Administration (US-FDA). Department of Health and Human Services. Center for Drug Evaluation and Research. Physicochemical and Structural (Q3) Characterization of Topical Drug Products Submitted in ANDAs. Draft Guidance for Industry. 2022. https://www.fda.gov/media/162471/download

FDA, 2022c. US-Food and Drug Administration (US-FDA). Center for Drug Evaluation and Research (CDER), Office of Regulatory Policy. Draft guidance on In Vitro Release Test Studies for Topical Drug Products Submitted in ANDAs. October 2022. Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/in-vitro-release-test-studies-topical-drug-products-submitted-andas.

García-Arieta A, Gordon J, Gwaza L, Merino V, Mangas-Sanjuan V. Regulatory Requirements for the Development of Second-Entry Semisolid Topical Products in the European Union. Pharmaceutics. 2023 Feb 10;15(2):601. doi: 10.3390/pharmaceutics15020601.

Holmgaard R, Nielsen JB, Benfeldt E. Microdialysis sampling for investigations of bioavailability and bioequivalence of topically administered drugs: current state and future perspectives. Skin Pharmacol Physiol. 2010;23(5):225-43.

N'Dri-Stempfer B, Navidi WC, Guy RH, Bunge AL. Improved bioequivalence assessment of topical dermatological drug products using dermatopharmacokinetics. Pharm Res. 2009 Feb;26(2):316-28. doi: 10.1007/s11095-008-9742-9.

Schmidt S, Banks R, Kumar V, Rand KH, Derendorf H. Clinical microdialysis in skin and soft tissues: an update. J Clin Pharmacol. 2008 Mar;48(3):351-64. doi: 10.1177/0091270007312152.

Shah VP, Rădulescu FŞ, Miron DS, Yacobi A. Commonality between BCS and TCS. Int J Pharm. 2016;509:35-40.

Shah VP, Yacobi A, Radulescu FS, Miron DS, Lane ME. A science-based approach to topical drug classification system (TCS). Int J Pharm. 2015;491:21–5.

USP, 2022. Pharmacopeial Forum. General Chapters. General Information. ⟨1724⟩ Semisolid Drug Products - Performance Tests. 2022;Pharmacopeial Forum 48(3).

USP, 2023. United States Pharmacopoeia - National Formulary (USP), 2023. Chapter 1724: Semisolid Drug Products - Performance Tests, United States Pharmacopeial Convention, Rockville, MD 20852.

Viziteu HM, Miron DS, Lupuliasa D, Rădulescu FȘ. The role of microstructural testing in the assessment of the in vitro release profiles from topical semisolid formulations. Farmacia 2024b;72(2):322-36. https://doi.org/10.31925/farmacia.2024.2.11.

Viziteu HM, Rădulescu FȘ, Dan M, Lupuliasa D, Miron DS. The development of in vitro release methodology for high strength topical semisolid formulations of anti-inflammatory drugs. Farmacia 2024a;72(1):78-86. https://doi.org/10.31925/farmacia.2024.1.7.

Yacobi A, Shah VP, Bashaw ED, Benfeldt E, Davit B, Ganes D, Ghosh T, Kanfer I, Kasting GB, Katz L, Lionberger R, Lu GW, Maibach HI, Pershing LK, Rackley RJ, Raw A, Shukla CG, Thakker K, Wagner N, Zovko E, Lane ME. Current challenges in

bioequivalence, quality, and novel assessment technologies for topical products. Pharm Res. 2014 Apr;31(4):837-46. doi: 10.1007/s11095-013-1259-1.

List of the published papers

Viziteu HM, Rădulescu FȘ, Dan M, Lupuliasa D, Miron DS. The development of in vitro release methodology for high strength topical semisolid formulations of anti-inflammatory drugs. Farmacia 2024a;72(1):78-86. https://doi.org/10.31925/farmacia.2024.1.7. https://farmaciajournal.com/wp-content/uploads/art-07-Viziteu_Radulescu_Miron_78-86.pdf

Viziteu HM, Miron DS, Lupuliasa D, Rădulescu FȘ. The role of microstructural testing in the assessment of the in vitro release profiles from topical semisolid formulations. Farmacia 2024b;72(2):322-36. https://doi.org/10.31925/farmacia.2024.2.11. https://farmaciajournal.com/wp-content/uploads/art-11-Viziteu_Radulescu_Miron_322-336.pdf