BJO
Brazilian Journal of Oncology
ORIGINAL ARTICLES CLINICAL ONCOLOGY

Bioequivalence study between two formulations of 10mg lenalidomide capsules in healthy male subjects under fasting conditions

Estudo de bioequivalência entre duas formulações de cápsulas de 10mg de lenalidomida em indivíduos saudáveis do sexo masculino sob jejum

Carlos Sverdloff[1], Vinicius Marcondes Rezende[1], Ligia de Cassia Val[2], Nelly Cristina Castro[3], Maria Elena Cedano Limón[3], Mohammad H. Badii[3], Sreekanth Kakarla[3], Murali Pendela[3], Lygia Bonnato[2]

ABSTRACT

Objective: To evaluate pharmaceutical bioequivalence between two formulations of 10mg lenalidomide capsules in healthy male subjects under fasting conditions. **Material and Methods:** An open label, monocentric, randomized, 2x2 crossover study in 32 healthy men under fasting conditions comparing two formulations of lenalidomide capsules. Analyte concentrations in human plasma were determined using a validated liquid chromatography with a tandem mass spectrometer detector method (UPLC-MS/MS). **Results:** Statistical analysis has determined geometric mean of test/reference ratio, confidence intervals, and power of the test to the pharmacokinetic parameters C_{max} and AUC_{0-t} as required by Anvisa resolution, the geometric mean ratio (90%CI) of the test drug/reference drug were 84.01 to 108.10 for C_{max} and 98.58 to 105.34 for AUC_{0-t}. Power of the test was 89.9% for C_{max} and 100.0% for AUC_{0-t}.

Keywords: Lenalidomide; Therapeutic equivalency; Antineoplastic agents.

1. ATCGen, Clinical operations - Campinas - SP - Brazil.
2. Eurofarma, Clinical operations - São Paulo - São Paulo - Brazil.
3. Avant Santé Research Center, Clinical operations - Mexico City - Nuevo León - Mexico.

Financial support: none to declare.
Conflicts of interest: The authors declare no conflict of interest relevant to this manuscript.
Correspondence author: Carlos Sverdloff.
E-mail: carsver@atcgen.com.br

Received on: June 15, 2023 | **Accepted on:** November 23, 2023 | **Published on:** December 29, 2023
DOI: https://doi.org/10.5935/2526-8732.20230424

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

RESUMO

Objetivo: Avaliar a bioequivalência farmacêutica entre duas formulações de cápsulas de lenalidomida 10mg em indivíduos saudáveis do sexo masculino em jejum. **Material e Métodos:** Estudo aberto, monocêntrico, randomizado, cruzado 2x2 com 32 homens saudáveis em jejum, comparando duas formulações de cápsulas de lenalidomida. As concentrações do analito no plasma humano foram determinadas usando uma cromatografia líquida validada com um método detector de espectometria de massa tandem (UPLC-MS/MS). **Resultados:** A análise estatística determinou a média geométrica da relação teste/referência, intervalos de confiança e poder do teste para os parâmetros farmacocinéticos C_{max} e AUC_{0-t} conforme exigido pela resolução da Anvisa, a razão média geométrica (IC90%) do medicamento em teste/medicamento de referência foram: 84,01 a 108,10 para C_{max} e 98,58 a 105,34 para AUC_{0-t}. O poder do teste foi de 89,9% para C_{max} e 100,0% para AUC_{0-t}.

Descritores: Lenalidomida; Equivalência terapêutica; Agentes antineoplásicos.

INTRODUCTION

The high cost of cancer drugs, especially in developing countries, makes it difficult for patients to access the best available treatment. With new generic options for this product, it is expected that prices will fall back to more accessible levels and thus provide an improvement in the quality of health of users. Studies have shown that the inclusion of generic drugs in the treatment of CML improves patient access to treatment. Generic medicines tend to cost less than their brand-name counterparts because they do not have to repeat animal and clinical (human) studies that were required of the brand-name medicines to demonstrate safety and effectiveness. Lower-cost generic drugs have been shown to increase the likelihood that patients take essential medications prescribed by their doctors and to improve patients' health outcomes.

Two formulations, typically test and reference, are considered bioequivalent, if the ranges are between 80% and 125% according to Anvisa's Resolution RE number 1170, dated April 19th, 2006,[1] which determines that two drugs will be considered bioequivalent if the extreme values of the 90% confidence interval of the ratio of geometric means (AUC0-t test/AUC0-t reference and C_{max} test/C_{max} reference) are greater than 80% and less than 125%.

Lenalidomide (IUPAC 3-(7-amino-3-oxo-1H-isoindol-2-yl)piperidine-2,6-dione), a thalidomide analogue, is an immunomodulatory (IMiD) and antineoplastic agent used in multiple myeloma therapy.[2] Multiple myeloma is a malignant B-cells neoplasia characterized by the excess of monotypic plasma cells in the bone marrow. Established for the first time as agents with antiangiogenic properties, thalidomide and the other IMiDs inhibit the production of interleukin (IL)-6, which is a growth factor for the proliferation of myeloma cells. Additionally, they activate apoptotic pathways through cellular death by means of caspases.[3]

In 2005, the FDA approved the marketing of 5mg and 10mg Revlimid® (Lenalidomide) capsules for the treatment of transfusion-depending anemia patients due to low risk myelodysplastic syndrome (MDS) associated to 5q deletion abnormality with or without additional cytogenetic abnormalities and multiple myeloma in combination with dexamethasone.[4,5] The European Medicines Agency (EMA) issued the authorization to market lenalidomide in 2007.[6] In Brazil, *Agência Nacional de Vigilância Sanitária - Anvisa,* issued a public opinion for the approval of Revlimid in 2018 for the treatment of patients with relapsed/refractory multiple myeloma who had received at least one prior treatment; and for the treatment of patients with transfusion-depending anemia derived from low to intermediate-1 risk myelodysplastic syndrome (MDS) associated to 5q deletion cytogenetic abnormality, with or without additional cytogenetic abnormalities.[7]

In 2020, Anvisa issued RDC 393/2020, which included lenalidomide therapeutic indications for the treatment of multiple myeloma, in combination with bortezomib and dexamethasone, for patients with no prior treatment; for the treatment of follicular lymphoma or previously treated patients with marginal zone lymphoma, in combination with rituximab (anti-CD20 antibody), and relapsed/refractory mantle cell lymphoma.[8]

As to the security profile, authors report that lenalidomide is well tolerated and that most usual adverse events include hematologic toxicity with controllable neutropenia and thrombocytopenia.[9,10]

The objective of this study was to verify whether the rate and extent of absorption of the 10mg lenalidomide immediate release capsule formulation

manufactured by Eurofarma Laboratórios S.A. are equivalent to those of the reference product, Revlimid®, when administered in one single-dose and under fasting conditions to adult healthy male subjects.

METHODS

Study formulations

The test drug - 10mg lenalidomide immediate release capsule -, was manufactured by Eurofarma Laboratórios S/A. The reference product used in the study was Revlimid® (10mg lenalidomide immediate release capsule), manufactured by Celgene Europe B.V. and registered in Brazil by Bristol-Myers Squibb Farmacêutica LTDA.

Study volunteers

Based on its mechanism of action and findings from animal studies, lenalidomide can cause embryofetal harm when administered to a pregnant female and is contraindicated during pregnancy. [11] For this reason, only male volunteers were considered for this trial.

Thirty-two Mexican adult male healthy volunteers willing to participate in the study were selected based on the protocol eligibility criteria 90 days before to the first study period. A sufficient number of eligible volunteers showed up in the research center facilities, and the 32 volunteers who fulfilled the protocol requirements were given information regarding the study and, after having their inquiries clarified and having decided to willingly take part in the study, each subject signed the Informed Consent Form (ICF) previously approved by the Avant Santé Research Center Research Committee (COFEPRIS 18 CI 19 019 021) along with the study protocol (approval CONBIOÉTICA-19-CEI-010-20160830). Initially, 32 study subjects were selected and randomized; all the subjects of the study completed the procedures.

Study design

Single-dose, randomized, open-label, two-treatment, two-sequence, two-period, crossover bioequivalence study of 10 mg lenalidomide capsules manufactured by Eurofarma Laboratórios S.A. *versus* Revlimid® (10mg lenalidomide immediate release capsule) manufactured by Celgene Europe B.V. and registered in Brazil by Bristol-Myers Squibb Farmacêutica LTDA in adult male healthy volunteers under fasting conditions.

Drug administration

Study subjects were kept in fasting conditions for 10 hours prior to the dose administration and at least 4 hours afterwards on each period. The study was conducted under fasting conditions as required by Anvisa.[12]

On each period of the study, a single dose (10mg each) of the test or the reference product was administered orally to the volunteers, in a seated position, with 200ml of water at room temperature, under fasting conditions. The washout period was 7 days.

Blood sampling

A total number of 19 blood samples were collected of each volunteer in each period in tubes with K_2EDTA. Blood samples (4ml each) were collected at timepoints 0.00h (before dose) and after administration at timepoints 0.25, 0.33, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00 and 24.00 hours.

Biological samples processing

After collection, blood samples were processed in refrigerated centrifuge at 3,000rpm for 10 minutes at 4±2ºC. The plasma obtained from the blood samples was transferred to two different cryogenic tubes (aliquot 1 and aliquot 2) previously identified and stored at a temperature below -50°C.

Lenalidomide quantification in plasma

Method validation

The validation of the bioanalytical method for quantification of Lenalidomide in human plasma using Lenalidomide-d5 as internal standard and K2EDTA as anticoagulant through extraction in solid phase (Strata-X® 33µm cartridge) and liquid chromatography coupled to mass spectrometry (UPLC-MS/MS) was performed in compliance with the acceptance criteria for selectivity, calibration curve, precision, accuracy, residual effect, matrix effect and stability test in solution and in the biological matrix.

Chromatographic conditions adopted for the validation and quantification of the study subjects' samples included the use of a Luna omega 1.6µm PS C18 50x2.1mm chromatographic column, at 35±2 ºC. Samples were kept at 5±4 ºC in the sampler. Mobile phase A used was Formic Acid 0.1% and mobile phase B was LC-MS grade Acetonitrile in an 87:13 v/v proportion. The injection volume was 3.0 µL and retention times were 1.07±0.3 min for the analyte and 1.05±0.3 for the internal standard, the running time being 2.10 minutes.

The method proved linear between concentrations of 2.044ng/ml to 1015.506ng/ml according to equation y = a + bx [1/x], where "y" is the response, "x" is the analyte concentration and "1/x" is the selected weight.

The lower limit of quantification (LLOQ) established for the method was 2.044ng/ml and validated quality control samples were 5.148ng/ml, 411.867ng/ml and 792.052ng/ml.

Stability

Stability analysis was carried out in plasma in concentrations of 5.148ng/ml and 792.052ng/ml and they complied with the acceptance criteria when the samples were subjected to 6 hours and 18 minutes at room temperature (approximately 25ºC) (short-term stability), for 2 days 8 hours and 38 minutes in autoinjector (5±4ºC) after sample extraction completion (post-processing stability) 4 freeze-and thaw cycles (freezing temperature: -70°C±15°C) and 106 long-term days.

Standard solutions

Reference standards: lenalidomide (TRC/Canada) was used as analyte and Lenalidomide-d5 (TRC/Canada) was used as internal standard for the preparation of the primary standard solutions in methanol HPLC grade. Working solutions were prepared using milli-Q water: Methanol (20:80 v/v) as eluent. All solutions were stored at a temperature between 2 and 8ºC.

Compounds quantification in biological samples

Compounds were extracted from human plasma samples and quantified through liquid chromatography coupled to mass spectrometry (LC-MS/MS) using the Xevo TQ-S/Acquity UPLC I-Class (Waters) spectrometer, equipped with positive electrospray (ESP+) ionization source, and the analyte and internal standard were detected using MRM with m/z transitions 260.21>149.11 and 265.23>151.13, respectively.

Table 1 shows a summary of the bioanalytical method.

Software used

Software Masslynx version 4.1 was used for calculating sample concentrations in the analytic phase.

Software *Phoenix WinNonlin*™ version 8.3 and *Sistema de Análise Estatística* (SAS®) version 9.4 were used to perform the statistical analysis.

RESULTS

Study population

The study began with 32 volunteers and ended with 32 adult healthy male volunteers between 18 and 42 years of age and BMI between 18.7 and 26.9kg/m^2, who complied with the inclusion and exclusion criteria set forth in the protocol.

Pharmacokinetics and statistical analysis

Pharmacokinetic parameters C_{max} and AUC_{0-t} were established using software *Phoenix WinNonlin*™ version 8.3 and *Sistema de Análise Estatística* (SAS®) version 9.4.

Pharmacokinetic parameters are shown in the table below.

Maximum concentration C_{max} obtained for the reference product Revlimid® was 195.309ng/ml in 1.5 hour. For the test product, lenalidomide, C_{max} of 186.120ng/ml occurred at 1.1 hour.

Figure 1 shows intermediate concentrations of lenalidomide for the 32 study subjects along collection times.

Tolerability/Safety analysis

Four adverse events were reported during 10mg study: 2 headaches, 1 vomit and 1 dyspepsia. No serious adverse events were reported; 3 events were classified as mild and 1 as moderate.

As to the relation with the study drug, 3 adverse events were classified as probably related and 1 as possibly related.

Table 1. Summary of the bioanalytical method.

Analyte	Lenalidomide
Internal Standard	Lenalidomide-d5
Biological Matrix	Human Plasma
Anticoagulant	EDTA
Linearity	2.044ng/ml to 1015.506ng/ml
Curve Equation	y = a + bx [1/x]
Lower Limit of Quantification (LLOQ)	2.044ng/ml
Low Quality Control (LQC)	5.148ng/ml
Medium Quality Control (MQC)	411.867ng/ml
High Quality Control (HQC)	792.052ng/ml
Post-processing Stability Time	2 days 8 hours 38 minutes
Freeze/thaw cycles	4 cycles
Short-term stability time	6 hours 18 minutes
Long-term stability time	106 days

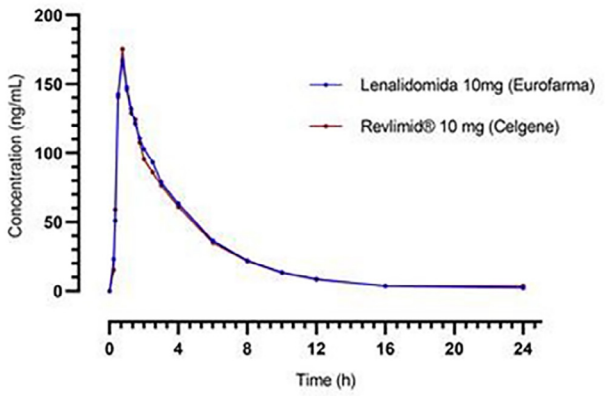

Figure 1. Lenalidomide intermediate concentrations along time for each formulation.

DISCUSSION

The study was planned and conducted according to the legal rules and regulations in force, obtaining pharmacokinetic parameters C_{max} and AUC_{0-t} which confidence interval values (90%) are within the acceptable limit for the ratio between the geometric means of the test and reference products (80-125%), according to the national legislation.[1]

The planned number of 32 adult healthy male subjects is consistent with studies published by other authors.[13,14] The population selected for the study was of male gender only, as recommended by the FDA guidelines[15] and consistent with Anvisa resolution RDC 735/2022.[16]

The clinical trial was conducted normally, and as mentioned above, there were no serious adverse events. Headache was the most frequent adverse event, contrary to the reports of other authors and the Anvisa panel, who stated neutropenia and thrombocytopenia as the most frequent adverse events.[9,17,18]

The 7-day washout period seemed adequate, as all base collection samples of the volunteers of the second period had a concentration below the Lower Quantification Limit (LQL).

As in other published works, the analytical technique selected for the study for lenalidomide quantification of in human plasma samples was LC-MS/MS.[19-21]

Lenalidomide was quantified in its unaltered form, as required by the Brazilian legislation.[22]

Both reference and test drugs showed a maximum plasma concentration C_{max} of 195.13ng/ml and 207.31ng/ml, respectively, consistent with those found in the literature.[13,14]

In countries with centralized health systems, such as in Brazil (*SUS: Sistema Único de Saúde*), the importance of generic drugs is based on maintenance of supply and negotiation with pharmaceutical companies. Being so, not only patients benefit from high-quality generic drugs: the savings for the Brazilian health care system from generic drugs is highly expressive. As health care costs continue to rise, it is important to continue to manufacture generic alternatives and make them available to patients, as this may help slow the increase in health care costs which are often passed along to patients.

CONCLUSION

Taking the results obtained in this study into account, it is concluded that the 10mg lenalidomide capsule test treatment (Eurofarma Laboratórios S/A) and the Revlimid® - 10mg lenalidomide capsule (Celgene Europe B.V.) reference treatment are bioequivalent regarding the absorption rate and extent, when administered under fasting conditions, as the criteria required by the Brazilian regulatory authority has been complied with (CI90% between 80-125%).

According to the statistical results, it can be concluded that both products tested in this study, namely 10mg Revlimid® capsules (reference product) and Lenalidomida Eurofarma (test product), meet the regulatory criteria for bioequivalence. Therefore, based on their biopharmaceutical performance, the test product can be considered interchangeable with the reference product, ensuring their comparability in terms of therapeutic effect and safety.

Based on adverse events and their severities, and clinical examination, electrocardiogram, and laboratorial assays, both products were considered well tolerated by the participants. The found adverse events' profile was in accordance with the reported in the literature and insert package reference product.

Finally, the use of generic drugs in the clinical practice must be encouraged and be an alternative for public health systems to reduce costs and keeping quality of offered treatment, once bioequivalence trials show interchangeably between generic and reference ones.

Table 2. Pharmacokinetic parameters (n=32).

Ratio (test/reference)	Geometric Mean (%)	CI (90%)	Test Power (%)	*p*-value (sequence)
C_{max} (ng/mL)	95.3	84.01 – 108.10	89.9	0.8631
AUC_{0-t} (ng.h.ml^{-1})	101.9	98.58 – 105.34	100.0	0.2633

AUTHORS' CONTRIBUTIONS

CS	Data analysis and interpretation, Manuscript writing, Final approval of the manuscript.
VMR	Manuscript writing, Final approval of the manuscript.
LCV	Conception and design, Provision of study materials or patient, Final approval of the manuscript.
NCC	Collection and assembly of data, Conception and design, Final approval of the manuscript.
MECL	Collection and assembly of data, Conception and design, Final approval of the manuscript.
MHB	Collection and assembly of data, Conception and design, Final approval of the manuscript.
SK	Collection and assembly of data, Conception and design, Final approval of the manuscript.
MP	Collection and assembly of data, Conception and design, Final approval of the manuscript.
LB	Conception and design, Final approval of the manuscript.

REFERENCES

1. Agência Nacional de Vigilância Sanitária (Anvisa). Resolução - RE 1.170, De 19 de abril de 2006. Guia para provas de biodisponibilidade relativa/bioequivalência de medicamentos. Brasília (DF): Anvisa; 2006.
2. National Library of Medicine (NIH). Compound Summary - CID 216326: Lenalidomide Information., Bethesda: NIH/PubChem.
3. Anderson KC. Lenalidomide and thalidomide: mechanisms of action--similarities and differences. Semin Hematol. 2005 Oct;42(4 Suppl 4):S3-S8.
4. Chen Y, Borthakur G. Lenalidomide as a novel treatment of acute myeloid leukemia. Expert Opin Investig Drugs. 2013 Mar;22(3):389-97.
5. US Food & Drug Administration (FDA). Drug Approval Package. 021880 - Revlimid (Lenalidomide) capsules. Celgene Corporation. Aproval Date: 27-Dec-2005 [Internet]. Washington, DC: FDA; 2005; [access in 2022 Nov 22]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/021880s000_RevlimidTOC.cfm
6. European Medicines Agency (EMA). EMA/13846/2020 - Revlimid lenalidomide. Aproval Date: 14-Jun-2007 [Internet]. Amsterdam: EMA; 2020; [access in 2022 Nov 22]. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/revlimid#authorisation-details-section
7. Agência Nacional de Vigilância Sanitária (Anvisa). Revlimid [Internet]. Brasília (DF): Anvisa; 2017; [access in 2022 Nov 22]. Available from: https://consultas.anvisa.gov.br/#/medicamentos/25351757160201560/?substancia=25181%20
8. Agência Nacional de Vigilância Sanitária (Anvisa). Lenalidomida: autorizadas novas indicações terapêuticas [Internet]. Brasília (DF): Anvisa; 2020; [access in 2022 Nov 22]. Available from: http://antigo.anvisa.gov.br/resultado-de-busca?p_p_id=101&p_p_lifecycle=0&p_p_state=maximized&p_p_mode=view&p_p_col_id=column-1&p_p_col_count=1&_101_struts_action=%2Fasset_publisher%2Fview_content&_101_assetEntryId=5893220&_101_type=content&_101_groupId=219201&_101_urlTitle=lenalidomida-autorizadas-novas-indicacoes-terapeutic-1&inheritRedirect=true
9. Armoiry X, Aulagner G, Facon T. Lenalidomide in the treatment of multiple myeloma: a review. J Clin Pharm Ther. 2008 Jun;33(3):219-26.
10. Fine HA, Kim L, Albert PS, Duic JP, Ma H, Zhang W, et al. A phase I trial of lenalidomide in patients with recurrent primary central nervous system tumors. Clin Cancer Res. 2007 Dec;13(23):7101-6.
11. Drugs.com. Lenalidomide pregnancy and breastfeeding warnings [Internet]. New York: Drugs.com; 2022; [access in 2022 Nov 22]. Available from: https://www.drugs.com/pregnancy/lenalidomide.html
12. Agência Nacional de Vigilância Sanitária (Anvisa). Lista 1 – Forma de administração (formas farmacêuticas de liberação imediata). Resolução - RE nº 1170, de 19 de abril de 2006, atualizada em 31/03/2022 [Internet]. Brasília (DF): Anvisa; 2022; [access in 2022 Jun 07]. Available from: https://www.gov.br/anvisa/pt-br/setorregulado/regularizacao/medicamentos/equivalencia-terapeutica/bioequivalencia/listas
13. Lee S, Hwang JG, Park SY, Lim HJ, Lee SW, Seo MH, et al. Single-dose comparative pharmacokinetics of two formulations of lenalidomide 25mg in healthy subjects: a randomized crossover study. Adv Ther. 2018 Feb;35(2):210-7.
14. Wang J, Qi L, Wang Z, Chen G, Liu C, Liu X, et al. Bioequivalence study of single-dose lenalidomide capsule vs. Revlimid((R)) capsule in healthy Chinese males. Cancer Chemother Pharmacol. 2018 Jul;82(1):159-64.
15. US Food & Drug Administration (FDA). Draft guidance on Lenalidomide [Internet]. Washington, DC: FDA; 2013; [access in 2022 Nov 23]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/psg/Lenalidomide_draft_Oral%20cap_RLD%2021880_RC11-13.pdf
16. Agência Nacional de Vigilância Sanitária (Anvisa). Resolução da Diretoria Colegiada - RDC nº 735, de 13 de julho de 2022. Dispõe sobre o controle da substância lenalidomida e de medicamento que a contenha, e dá outras providências. Brasília (DF): Anvisa; 2022.

17. Agência Nacional de Vigilância Sanitária (Anvisa). VigiMed - Eventos Adversos [Internet]. Brasília (DF): Anvisa; 2022; [access in 2022 Nov 22]. Available from: https://www.gov.br/anvisa/pt-br/acessoainformacao/dadosabertos/informacoes-analiticas/notificacoes-de-farmacovigilancia

18. Gay F, Hayman SR, Lacy MQ, Buadi F, Gertz MA, Kumar S, et al. Lenalidomide plus dexamethasone versus thalidomide plus dexamethasone in newly diagnosed multiple myeloma: a comparative analysis of 411 patients. Blood. 2010 Feb;115(7):1343-50.

19. Ranganathan P, Gunasekaran V, Singhvi I, Ansari MJ. Development and validation of Lenalidomide in human plasma by LC-MS/MS. Saudi J Biol Sci. 2019 Nov;26(7):1843-7.

20. Iqbal M, Wani TA, Khalil NY, Darwish IA. Development and validation of ultra-performance liquid chromatographic method with tandem mass spectrometry for determination of lenalidomide in rabbit and human plasma. Chem Cent J. 2013;7(1):7.

21. Hasnain MS, Rao S, Singh MK, Vig N, Gupta A, Ansari A, et al. Development and validation of LC-MS/MS method for the quantitation of lenalidomide in human plasma using Box-Behnken experimental design. Analyst. 2013;138(5):1581-8.

22. Agência Nacional de Vigilância Sanitária (Anvisa). Lista 2 - Analito para Estabelecimento da Biodisponibilidade Relativa/Bioequivalência. Resolução - RE nº 1170, de 19 de abril de 2006, atualizada em 31/03/2022 [Internet]. Brasília (DF): Anvisa; 2022; [access in 2022 Jun 07]. Available from: https://www.gov.br/anvisa/pt-br/setorregulado/regularizacao/medicamentos/equivalencia-terapeutica/bioequivalencia/listas