dx.doi.org/10.14227/DT290222PGC2

Pharmaceutical Equivalence of Losartan Potassium Tablets in Argentina

María A. Varillas[1], Marta I.V. Brevedan[1], and Noelia L. Gonzalez Vidal[1,2*]

[1]Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina.
[2]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina.

e-mail: nlgvidal@uns.edu.ar

ABSTRACT

Losartan potassium (LOK) is an antihypertensive agent from the group of selective angiotensin AT1 receptor antagonists, widely used in the form of tablets for oral administration. The available data for BCS classification are confusing, though class III or IV are most likely. The quality attributes of solid oral dosage forms of LOK immediate-release tablets (50 mg) available in the Argentine pharmaceutical market were evaluated according to the Argentine Pharmacopoeia and United States Pharmacopeia (i.e., storage conditions information, price per tablet, average weight, assay, uniformity of dosage units, hardness, disintegration time, and in vitro dissolution). The dissolution efficiency (DE) results were compared using one way analysis of variance. All evaluated samples were within the acceptable limits for disintegration time, hardness, assay, uniformity of dosage units, and in vitro dissolution (in Stage 1). A statistically significant difference in DE was recorded for samples C, D, E, F, and G compared to the reference formulation (sample H). These samples had higher DE values than the reference; there were no statistically significant differences between the samples. Therefore, the evaluated samples of LOK from the Argentine market can be considered pharmaceutical equivalents.

KEYWORDS: In vitro dissolution, losartan potassium tablets, pharmaceutical equivalence, quality control.

INTRODUCTION

Losartan potassium (LOK) was developed by DuPont-Merck laboratories for hypertension treatment (*1, 2*). This active pharmaceutical ingredient (API) is a potent non-peptide angiotensin II receptor (type AT1) antagonist. When administered orally, LOK is partially (~14% of the ingested dose) transformed into its 5-carboxylic acid metabolite, which is more potent than LOK and has an extended pharmacological effect (*1, 2*). Peak plasma concentrations of LOK and its active metabolite are reached approximately 1 to 3 hours after oral administration (*3*).

The Biopharmaceutical Classification System (BCS) data for LOK provided in published literature are contradictory. LOK has been shown to be a class I drug based on the supporting evidence from Takagi et al., whereas Gunda et al have described LOK as a class II drug with no supporting evidence (*4*, *5*). Kasim et al. reported that LOK has high solubility based on the dose number D_o and low permeability, possibly related to an efflux mechanism, with no association to a specific BCS Class (*6*). Other studies have shown that LOK belongs to class III or class IV; however, Souza et al. indicated that those studies were conducted under conditions not provided by the BCS

*Corresponding author

scheme (*7–10*). Nevertheless, Souza et al also concluded that LOK could be a class IV drug based on shake-flask methodology for solubility measurement or class III, considering the intrinsic dissolution results (*10*).

The Argentine pharmaceutical market is depicted by reference and multisource products, with absence of generic medicine in the strict sense (i.e., as they are recognized in other countries). In this scenario, patients in Argentina interchange formulations based on price and availability of a product, and LOK tablets are no exception. This is why establishing pharmaceutical equivalence between products is needed. For this purpose, all evaluated products must meet the same quality standards, such as identity and drug content, dose uniformity, and in vitro dissolution behavior, and have consistent information about storage conditions (*11, 12*).

The in vitro dissolution test (as a single point estimate) is an effective method to ensure product quality and assess pharmaceutical equivalence with the reference product, especially for immediate-release formulations containing an API with high solubility and/or permeability (*13*). Comparison of dissolution profiles appears to be more accurate than the point estimate approach for APIs with low solubility and/or permeability(*14, 15*).

This study aims to compare solid oral immediate-release pharmaceutical products containing 50 mg of LOK, approved for commercialization in the Argentine market, to evaluate critical quality attributes and pharmaceutical equivalence with the reference product according to local and international guidelines.

MATERIALS AND METHODS

The LOK reference standard (99.9% calculated on the dried basis, 0.4% water content) was purchased from Saporiti (Argentina).

Eight different brands of immediate-release tablets containing LOK (50 mg) were purchased in Argentine pharmacies located in Bahía Blanca. The multisource formulations (manufactured in Argentina) were randomly labeled as A to G, and the reference product (imported from Brazil) was labeled as H.

Distilled water was obtained from our own laboratory and used as the dissolution medium.

Equipment

Equipment used for quality control tests included the following: Varian Cary 50 Conc spectrophotometer (Varian Instruments, Australia) for API quantification during assay and dissolution studies; Scout DGM02 and EGMO2 (Scout Electronics, Argentina) for hardness and disintegration time measurements, respectively; Erweka DT60 (Erweka GmbH, Germany) dissolution tester; and Acculab ALC-210.4M electronic analytical balance (Acculab, USA).

Quality Assessments

WHO Technical Reports state that the instructions for use and storage specifications, detailed on pharmaceutical product packages, are essential to guarantee the interchangeability of medicines (*12*). In this sense, to verify compliance with local legislation and WHO indications, the information included in labels and patient leaflets were compared (*11, 12, 16*).

Ten randomly chosen tablets of each sample product were individually weighed for weight

variation analysis. The mean weight and standard deviation (SD) were calculated.

Hardness was evaluated for 10 individual tablets of each sample, applying enough force to cause fracture along the diameter (*16*). Results were expressed as mean ± SD in kilopounds (kp). Disintegration time was assessed with six tablets of each product in distilled water at 37.0 ± 2.0 °C. Results were expressed as the maximum time needed for complete disintegration in seconds. After 30 minutes, each tablet should completely disintegrate, according to Argentine Pharmacopeia (*16*).

For the drug content assay, 20 tablets of each sample were weighed and crushed into a powder. Powder corresponding to 50 mg of LOK was weighed and dissolved in 100 mL of distilled water. The solution was filtered through a 0.45-µm pore size nylon membrane (Gamafil, Argentina) and diluted. LOK concentration was determined by UV spectrophotometry at 204 nm. The standard calibration curve was constructed for this purpose (y = 0.0962x + 0.0044; R^2 = 0.9994: concentration range 2.0–9.0 µg/mL) (*17, 18*). The same method was used to evaluate uniformity of dosage units, though applied over 10 individual tablets of each product. Assay results were compared using a one-way analysis of variance (ANOVA).

The dissolution test was carried out using a USP apparatus 2 (paddle) rotating at 50 rpm, with 900 mL of distilled water (37.0 ± 0, 5°C), according to USP (*19*). Aliquots of 10 mL were taken at 30 minutes, filtered through a 0.45-µm pore size nylon membrane (Gamafil) and subsequently diluted. To determine the LOK amount dissolved in the aliquot, the absorbance was measured (256 nm) and compared with the calibration curve (y = 0.0258x + 0.0171; R^2 = 0.9993; concentration range 5.0–60.0 µg/mL LOK). Pharmacopeial specifications state that not less than 75% of the declared amount of LOK must dissolve within 30 minutes (*19*). The same conditions were applied to construct the dissolution profiles, with sampling points at 5, 10, 15, 30, and 45 minutes. Dissolution efficiency (DE) was calculated for each profile, and the results were compared using ANOVA (*20*).

RESULTS AND DISCUSSION

The information included on labels (primary and secondary packaging) and patient leaflets was analyzed according to national and international criteria. The concept of interchangeability is applicable to the API and dosage form, including the directions for use and storage, which are particularly critical for stability and shelf life (*11, 12, 16*).

USP states for LOK tablets to “store in tightly closed containers, protected from light, at controlled room temperature. The term “controlled room temperature” is defined as ”a temperature thermostatically maintained between 20 and 25 °C, allowing deviations between 15 and 30 °C, experienced in pharmacies, hospitals, and warehouses” (*19*). The analyzed labels and leaflets showed differences in the information provided about storage conditions; however, all samples consistently specified the condition that temperature should be under 30 °C (Table 1). Regarding the USP recommendation about protection from light, only products A and H mentioned this issue. Samples A, B, D, and F specified to store in a dry place. Only the reference sample mentioned all the conditions established in the corresponding monograph. It is essential to achieve harmonization of the information presented on labels and leaflets, and its control by the authorities, for the correct interpretation by patients and health professionals.

Table 1. Product Information and Results of Physical Quality Control Tests of LOK (50 mg) Tablets

Product	Price[a]	Storage Conditions[b]	Weight (mg), Mean ± SD	Hardness (kp), Mean ± SD	Disintegration Time (s)[c]
A	40.70	Store in a dry place at a temperature between 15 and 30 °C, protected from light.	152.6 ± 1.9	6.0 ± 0.4	464
B	42.02	Store in a dry place at a temperature below 30 °C.	151.4 ± 3.0	9.6 ± 0.3	585
C	32.93	Store at a temperature below 30 °C.	199.1 ± 2.4	5.6 ± 0.3	577
D	18.18	Store in a dry place at room temperature of 25°C, allowed variation between 15 and 30 °C.	257.3 ± 2.4	9.2 ± 0.6	609
E	41.87	Store the product at a temperature no higher than 30 °C.	156.2 ± 3.1	5.2 ± 0.2	1168
F	43.24	Store in a cool and dry place, preferably between 15 and 30 °C.	290.1 ± 2.4	12.0 ± 0.3	497
G	53.79	Store up to 30 °C.	158.0 ± 2.9	5.2 ± 0.4	691
H (Ref.)	64.97	Keep the container closed and protected from light at temperature below 30 ° C.	154.7 ± 1.2	7.1 ± 0.4	666

[a]Price in Argentine pesos, at the time of analysis, per tablet.
[b]Information presented in labels and leaflets.
[c]Maximum time needed for complete disintegration of evaluated tablets.

Table 2. Assay, Uniformity, and Dissolution Results for LOK (50 mg) Tablets

Product	Assay[a] (Mean ± SD)	Uniformity of Dosage Units, % (Range / RSD)[b]	Dissolution Test (S1 Stage), % (Range / RSD)[c]	Dissolution Efficiency (Mean ± SD)
A	97.2 ± 0.4	95.9–99.1 / 1.2	86–96 / 3.7	71.1 ± 4.4
B	100.6 ± 2.4	100.6–105.7 / 1.7	96–100 / 1.7	70.6 ± 1.4
C	98.2 ± 0.3	97.1–100.5 / 1.3	94–103 / 3.8	74.1 ± 3.3
D	99.5 ± 1.3	97.2–99.3 / 0.8	97–102 / 2.1	76.3 ± 1.8
E	98.7 ± 2.9	98.1–103.7 / 2.3	97–106 / 3.4	79.9 ± 4.3
F	103.7 ± 0.4	102.2–103.9 / 0.7	80–98 / 7.6	77.1 ± 3.9
G	96.4 ± 0.0	94.0–98.2 / 1.7	84–99 / 7.1	76.0 ± 5.3
H (Ref.)	103.7 ± 0.1	102.8–104.1 / 0.4	90–100 / 3.6	68.9 ± 1.9

[a]Specification for acceptance: 95.0–105.0%.
[b]Specification for acceptance: range 85.0–115.0%; RSD < 6%.
[c]USP specification for acceptance at S1 Stage: no unit dissolves less than 75% (Q) + 5%, in 30 min.
RSD: relative SD; Q: amount of dissolved active pharmaceutical ingredient, specified in the individual monograph, expressed as a percentage of labeled content of the dosage unit.

Moreover, it should be noted that product C reported 50 mg of losartan as the labeled amount, instead of 50 mg of LOK. Product E was the only one that reported the date of manufacture. Three of the products claimed to be gluten free (C, E, and G), and C declared that the starch present in the formulation is corn starch. All products were scored tablets, except for C and D. Products B, C, E, and G reported the qualitative composition of the formulation, whereas A, D, F, and H declared both the qualitative and quantitative formula.

LOK multisource products A, B, E, and F had similar prices and were approximately 33% cheaper than the reference H, and sample D cost was 72% less (Table 1). In Argentina, economics is the most common reason for the extensive use of multisource formulations and decisions to interchange them by patients.

Table 1 shows the results of the physical quality control tests, carried out as the Argentine Pharmacopoeia recommends. The average weight of the tablets ranged from 151.4–290.1 mg (samples B and F, respectively). Products A, B, E, and G had an average weight similar to the reference, and F doubled this value. Sample A was the only formulation that reported the average tablet weight in the quantitative composition of the product leaflet. This range of results could be justified by differences in the composition and physical dimensions of each formulation, which are characteristic of each manufacturer and not necessarily related to variations in API content or dissolution performance.

Hardness represents the force required for tablets to fail (break) in a specific plane, and it is desirable that no tablet has hardness values below 2.0 kp. Mean hardness values ranged from 5.2–12.0 kp for all analyzed products (Table 1).

Finally, all samples met the pharmacopeial requirement of disintegration time (less than 30 min), with values ranging from 7.7–19.5 minutes (samples A and E respectively) (Table 1).

Table 2 depicts the results obtained for assay, uniformity of dosage units, and dissolution tests. All formulations complied with assay, with results ranging from 96.4 ± 0.0 to 103.7 ± 0.4. Statistical differences were recorded between the reference (H) and samples A, C, E, and G ($p < 0.01$) and between H and D ($p < 0.05$); however, no significant differences were observed between H and F or B. All formulations complied with specifications for uniformity of dosage units.

Regarding the in vitro dissolution test, as a critical quality control attribute, all samples met the specifications at Stage 1. The dissolution profiles of LOK tablets in distilled water are shown in Figure 1. In this particular case, differences were observed in the dissolution behavior of the evaluated samples. At 10 minutes, all samples dissolved less than an 80% of the API. Samples B, C, and H did not exceed 80% dissolved at 15 minutes, whereas the other samples did. All samples exceed 85% dissolved within 30 minutes, so they could be considered “rapidly dissolving” formulations.

Significant differences were detected when comparing the DE results of the reference formulation H (68.9 ± 1.9) with respect to samples C, D, E, F, and G ($p < 0.01$); however, these samples had higher DE values than the reference but there were no statistically significant differences between them. As shown in Figure 1, dissolution results for sample H were the lowest for the first 25 minutes of the test (i.e., sample H exhibited

the lowest dissolution rate). This can be partially explained by the composition of this formulation (Table 3). Sample H did not contain any of the superdisintegrants that were present in the remaining formulations (croscarmellose sodium, sodium starch glycolate, or crospovidone). Indeed, sample H contained conventional disintegrants as cellulose microcrystalline, hydroxypropyl cellulose, and carnauba wax, which could delay the dissolution process.

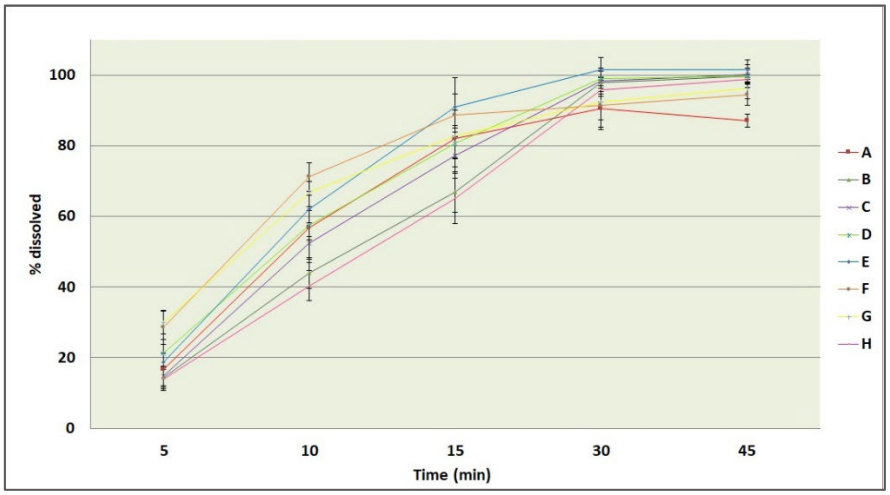

Figure 1. Mean ± SD percentage of labeled amount dissolved for LOK (50 mg) tablets. Sample H is the reference formulation.

Table 3. Qualitative Composition of Excipients in LOK (50 mg) Tablets

Excipient	A[a]	B	C	D[a]	E	F[a]	G	H (Ref.)[a]
Cellulose, Microcrystalline[b]	+	+	+	+	+	+	+	+
Colloidal Silicon Dioxide	+	-	+	-	+	+	+	-
Coloring agents	+	+	+	-	+	+	-	-
Croscarmellose sodium	-	-	+	-	+	+	-	-
Crospovidone	+	-	-	-	-	-	-	-
Hydroxypropyl cellulose	-	-	-	-	-	-	-	+
Hypromellose[b]	-	+	-	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Magnesium Stearate	+	+	+	+	+	+	+	+
Maltodextrin	-	-	-	-	+	-	-	-
Polyvinyl Alcohol[b]	+	-	+	-	-	-	-	-
Polyethylene glycol	+	+	+	+	-	+	+	-
Povidone	-	-	-	-	-	+	-	-
Propylene glycol	-	-	-	-	-	+	-	-
Saccharin sodium	-	-	-	-	-	+	-	-
Simethicone	+	-	-	+	-	-	-	+
Sodium starch glycolate	-	+	-	+	-	-	+	-
Starch	+	-	+	-	-	-	-	-
Talc[b]	+	-	+	+	-	+	-	-
Titanium dioxide[b]	+	+	+	+	+	+	+	+
Triacetin	-	+	-	-	+	-	-	-
Wax, Carnauba	-	-	-	-	-	-	-	+

[a]*Information presented in label and/or leaflet.*
[b]*This excipient has multiple functions.*
+ indicates present, - indicates absent.

CONCLUSION

National and international regulations were applied for the analysis of LOK tablets, including reference and multisource products, available in pharmacies of Argentina. The results show that the formulations included in the present study met the requirements in terms of assay, hardness, disintegration time, uniformity of dosage units, and dissolution test. When comparing the dissolution profiles, significant differences were recorded between the reference formulation and certain evaluated samples, although these differences would not have clinical impact. Therefore, the evaluated samples of LOK (50 mg) from the Argentine market can be considered pharmaceutical equivalents. Despite the established pharmaceutical equivalence, a biowaiver could not be established because there is no consensus about the BCS class of LOK.

FUNDING

The authors are grateful to Universidad Nacional del Sur for the financial support (PGI 24/B306).

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

REFERENCES

1. Barreiro, E. J.; Fraga, C. A. M. *Química Medicinal: As Bases Moleculares da Ação dos Fármacos,* 3rd Ed.; Artmed Editora: São Paulo, Porto Alegre, 2014.
2. Korolkovas, A. *Dicionário terapêutico Guanabara*, Ed. 2006/2007; Guanabara Koogan: Rio de Janeiro, 2007.
3. Kirch, W.; Horn, B.; Schweizer, J. Comparison of angiotensin II receptor antagonists. *Eur J Clin Invest*. **2001**, *31* (8), 698–706. DOI: 10.1046/j.1365-2362.2001.00871.x.
4. Takagi, T.; Ramachandran, C.; Bermejo, M.; Yamashita, S.; Yu, L. X.; Amidon, G. L. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol Pharm*. **2006**, *3* (6), 631–643. DOI: 10.1021/mp0600182.
5. Gunda, R. K.; Manchineni, P. R. Development and evaluation of losartan potassium sustained release tablet formulations. *World J. Curr. Med. Pharm. Res*. **2019**, *1* (1), 25–31.
6. Kasim, N. A.; Whitehouse, M.; Ramachandran, C.; Bermejo, M.; Lennernäs, H.; Hussain, A. S.; Junginger, H. E.; Stavchansky, S. A.; Midha, K. K.; Shah, V. P.; Amidon, G. L. Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Mol Pharm*. **2004**, *1* (1), 85–96. DOI: 10.1021/mp034006h.
7. Cristofoletti, R. [Comparative analysis of the predictive capacity of biowaiver models based on the SCB or SCBBD regarding the outcome of a bioequivalence study.] [in Portuguese] Thesis (Master's degree). State University of Londrina, PR, Brazil, 2013.
8. Ramirez, E.; Laosa, O.; Guerra, P.; Duque, B.; Mosquera, B.; Borobia, A. M.; Lei, S. H.; Carcas, A. J.; Frias, J. Acceptability and characteristics of 124 human bioequivalence studies with active substances classified according to the Biopharmaceutic Classification System. *Br J Clin Pharmacol*. **2010**, *70* (5), 694–702. DOI: 10.1111/j.1365-2125.2010.03757.x.
9. Bergström, C. A. S.; Andersson, S. B. E.; Fagerberg, J. H.; Ragnarsson, G.; Lindahl, A. Is the full potential of the Biopharmaceutics Classification System reached? *Eur J Pharm Sci.* **2014**, *57*, 224–231. DOI: 10.1016/j.ejps.2013.09.010.
10. Souza, J. B.; Souza, J.; Castro, L. M. L.; Siqueira, M. F.; Leal Savedra, R. M. L; Silva-Barcellos, N. M. Evaluation of the losartan solubility in the biowaiver context by shake-flask method and intrinsic dissolution. *Pharm. Dev. Technol*. **2019**, *24* (3), 283–292. DOI: 10.1080/10837450.2018.1472610.
11. ANMAT 5904 Regulation: *Definitions and general guidelines on how the information should be included in the leaflets of pharmaceutical products whose condition of sale is under prescription*; Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT): Buenos Aires, 1996.

http://www.anmat.gov.ar/webanmat/NORMATIVA/NORMATIVA/MEDICAMENTOS/DISPOSICION_ANMAT_5904-1996.PDF (accessed December 3, 2021).
12. WHO Expert Committee on Specifications for Pharmaceutical Preparations. *Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability*; WHO Technical Report Series, No. 1003, Annex 6; World Health Organization, 2017; pp 181-236.
13. Dressman, J.; Kramer, J.; Eds. *Pharmaceutical Dissolution Testing*. Marcel Dekker: New York, 2005.
14. Sathe, P. M.; Tsong, Y.; Shah, V. P. In vitro dissolution profile comparison: statistics and analysis, model dependent approach. *Pharm. Res*. **1996**, *13* (12), 1799–1803. DOI: 10.1023/A:1016020822093.
15. Shah, V. P.; Tsong, Y.; Sathe, P.; Williams, R. L. Dissolution profile comparison using similarity factor, *f*2. *Dissolut. Technol*. **1999**, *6* (3), 15. DOI: 10.14227/DT060399P15.
16. *Argentine Pharmacopeia*, 7th ed. Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT): Buenos Aires, 2013. www.argentina.gob.ar/anmat/farmacopea-argentina/libro (accessed December 3, 2021)
17. Tsvetkova, D. D.; Ivanova, S. A. Application of UV-spectrophotometric method for determination of losartan potassium in tablets. *Indo Am. J. Pharm. Sci*. **2018**, *5* (8), 8393–8401. DOI: 10.5281/zenodo.1411659.
18. Bonfilio, R.; Favoretto, L. B.; Pereira, G. R.; Azevedo, R. C. P.; Araújo, M. B. Comparative study of analytical methods by direct and first-derivative UV spectrophotometry for evaluation of losartan potassium in capsules. *Braz J Pharm Sci*. **2010**, *46* (1), 147–155. DOI: 10.1590/S1984-82502010000100017.
19. *The United States Pharmacopeia and National Formulary USP 41–NF 36.* The United States Pharmacopeial Convention, Inc.: Rockville, MD, 2018.
20. Khan, K. A. The concept of dissolution efficiency. *J Pharm Pharmacol*. **1975**, *27* (1), 48–49. DOI: 10.1111/j.2042-7158.1975.tb09378.x.