



Comparative dissolution and polymorphism study of clopidogrel bisulfate tablets available in Argentina

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ABSTRACT

Clopidogrel bisulfate (CLP) is an antiplatelet agent which exists in several solid forms. This study aimed to survey the dissolution performance of eight 75 mg CLP products available in Argentina, including the innovator brand (Plavix[®]) and the solid form of CLP in tablets. Dissolution behavior was evaluated by the United States Pharmacopeia (USP) 39 method (paddle, pH 2.0). The polymorphic state of CLP was investigated using powder X-ray diffraction (PXRD). The release profiles were compared using the similarity factor f_2 , bootstrap-based f_2 , dissolution efficiency (DE), medium dissolution time (MDT), and several kinetic models. All products met the USP specification at 30 minutes but their release characteristics varied widely. Statistical comparison of profiles indicated that only two products were found to be similar ($p > 0.05$) to Plavix[®] in terms of DE and MDT values. Kinetically, Plavix[®] and two products are fit the Weibull model, although they differed in model parameters. PXRD revealed that six products and Plavix[®] contained CLP form II and one contained CLP form I. The USP method was found to be convenient for the dissolution testing of CLP products, revealing differences in rate and extent of dissolution, which could raise questions about the interchangeability of the evaluated products.

INTRODUCTION

Governmental health policy in many countries throughout the world encourages the prescribing of generic or multisource products as an alternative to branded ones, primarily to promote access to medicines for low-income patients. However, it must be assured that generic and multisource products are quality medicines, which exhibit therapeutic equivalence and are switchable with the innovator or reference product.

It is reported that various substandard medicines are freely accessible in global markets (Reddy *et al.*, 2014). Consequently, drug resistance and treatment failure are commonly reported in emerging markets, such as in Asian and African countries (Reddy *et al.*, 2014). The Americas and Argentine do not escape this problem since many multisource products were found to be *in-vitro* inequivalent to the innovator ones (Kassuha *et al.*, 2009; Löbenberg *et al.*, 2012; Simionato *et al.*, 2018; Sperandeo and Kassuha, 2009), and at least in the case of some drugs [Biopharmaceutical Classification System (BCS) Class II, highly permeable and poorly soluble], significant differences in the dissolution profiles will result in differences in bioavailability since dissolution is the rate-determining step of the absorption process (Ruiz *et al.*, 2012).

Clopidogrel bisulfate (CLP, CAS 120202-66-6, Fig. 1) is an antiplatelet agent that is widely used for the reduction of atherosclerotic events in patients with stroke, myocardial infarction, cardiovascular disease, peripheral arterial disease, and acute coronary syndrome (Di Girolamo *et al.*, 2010; Plosker *et al.*, 2007). CLP, in combination with aspirin, is also a routine component of the clinical management

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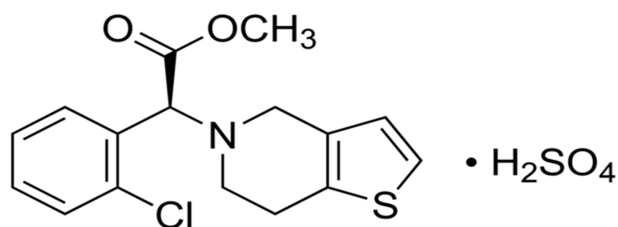


Figure 1.

Figure 1. Chemical structure of (S)-(+)-CLP.

of patients after acute coronary syndrome (Di Girolamo *et al.*, 2010). CLP is categorized as a BCS class II drug; this is primarily due to its poor solubility in intestinal pH and the main site of its absorption, resulting in very low (<50%) oral bioavailability (El-Laithy *et al.*, 2018). In addition, CLP exists in at least six crystalline forms and one amorphous form. Among these forms, only polymorphs I and II are used in pharmaceutical formulations (Bousquet *et al.*, 2003; Lestari *et al.*, 2010; Lifshitz-Liron *et al.*, 2003; Setiawati, 2011) since they have the same therapeutic indications and it is allowed to use alternate polymorphic forms as long as the criteria of pharmaceutical equivalence and bioequivalence are met (Setiawati, 2011). For both these polymorphs, only the dextrorotatory isomer (Fig. 1) exhibits activity on the platelet aggregation, whereas the levorotatory isomer is less active and poorly tolerated (Koradia *et al.*, 2004).

Due to its BCS class, low bioavailability, and indications of use, CLP generic and multisource products must demonstrate pharmaceutical equivalence and bioequivalence with the innovator product Plavix® in many countries. In Argentina, both the original brand (Plavix®, Sanofi-Aventis) and several multisource products of CLP have marketing authorization at a strength of 75 mg (expressed as clopidogrel base). According to the National Administration of Medicines, Food, and Medical Technology (ANMAT) of Argentina, each CLP multisource product must be shown to be pharmaceutical equivalent and bioequivalent to Plavix® (ANMAT, 2017) in order to be interchangeable with it. To date, however, only one product has been recognized by the ANMAT (ANMAT, 2019) as bioequivalent to Plavix®, and no studies evaluating the pharmaceutical equivalence of CLP tablets of the national market were reported. The absence of such studies is a potential risk for the public health, since in Argentine multisource products are allowed to be switched during dispensing in the pharmacy, except for some therapeutic groups such as antiepileptic and immunosuppressant drugs, provided that the patient agrees on the substitution (Congress of the Argentine Republic, 2002). To rely on a safe switch ability of Plavix® by a CLP multisource product is necessary to assure that it exhibits pharmaceutical equivalence and bioequivalence with the innovator. Thus, the objective of the present study was to evaluate and compare critical quality attributes, including *in-vitro* dissolution performance and polymorphic forms of CLP, of eight 75 mg CLP tablet products available in Argentina and to assess their pharmaceutical equivalence, since patients with coronary artery disease and cerebrovascular accidents use CLP tablets daily as part of their antiplatelet regimen, and differences in formulation could influence the release characteristics of the dosage

forms and even the bioavailability questioning the interchangeability of the products. To carry this out, the dissolution method specified in the United States Pharmacopeia (USP) 39 monograph for CLP tablets (United States Pharmacopeia (USP), 2016a) was used (since there is no monograph for CLP in the Argentine Pharmacopeia), and powder X-ray diffraction (PXRD) analyses were carried out to identify the polymorphic state of CLP in powdered tablets of the selected products.

MATERIALS AND METHODS

Materials

Eight different CLP tablet products were included in the study, which were designated with the Roman numbers I–VIII. Of these, Plavix® was named product I and taken as the reference. The products were purchased from pharmacies in Córdoba City (Argentina) or kindly donated by a local distributor and analyzed before their expiration dates, which were similar for all products. The CLP USP reference standard (Lot G1K326) was obtained from the USP (Rockville, MD). Analytical grade hydrochloric acid was purchased from Cicarelli S.A. (Argentina). All the other chemicals and reagents were of analytical grade and acquired commercially.

Evaluation of labels and prospects

A comparison of the information included on primary and secondary packaging's (boxes) and patient information leaflets (PILs) of the tested products was carried out to confirm compliance with both the Argentine regulations on information to be included in PILs of pharmaceutical products whose condition of sale is under prescription (ANMAT, 1996) and the USP 39 specification regarding product packaging and storage conditions, namely "preserve in well-closed containers and store at controlled room temperature" (United States Pharmacopeia (USP), 2016a).

Weight variation

Twelve tablets belonging to each product were weighed individually with an electronic balance (Shimadzu AEG 220, Kyoto, Japan), and the average weight was calculated. Then, the individual weight of each tested tablet was compared to the average weight. The tablet batches passed the test if no more than two of the individual weights recorded deviated from the average weight by more than $\pm 7.5\%$ (percentage limit for tablets with average weights of 130–324 mg) and with none deviating by twice $\pm 7.5\%$ (United States Pharmacopeia (USP), 2016b).

Dissolution studies

All dissolution studies were conducted on a Hanson SR6 dissolution tester (Hanson Research, Chatsworth, CA). The experimental conditions were as follows: USP Apparatus 2, 1,000 ml of deaerated pH 2.0 hydrochloric acid buffer (which was deaerated by heating to 40°C, vacuum filtering using a 0.45 μm nylon filter and stirring vigorously under vacuum for 5 minutes), $37.0 \pm 0.5^\circ\text{C}$, and 50 rpm. Samples (3 ml) were withdrawn manually (without medium replacement) at 7.5, 10, 15, 20, 30, and 40 minutes using a 5 ml syringe, and the amounts withdrawn were adjusted in the calculations. To filter the aliquots, Teflon filters (Hanson Research, P/N 27-101-074, 10 μm), fitted to the stainless steel cannulas of the dissolution, were

used. The dissolution samples were kept in screw-capped test tubes, and the absorbance was measured, in duplicate, at 240 nm using a UV-visible spectrophotometer (Perkin Elmer Lambda 25 Cambridge, UK). The drug concentrations were determined using a seven-point calibration curve (obtained with the CLP reference substance in the dissolution medium). The dissolution profiles were represented as the cumulative percentages of the amount of drug released at each sampling interval, with each profile being the average of 12 individual tablets.

Data analyses

The comparison of profiles was carried out using model-independent and model-dependent approaches.

Model-independent methods

As model-independent methods, the similarity factor (f_2), dissolution efficiency (DE), and medium dissolution time (MDT) were calculated.

The f_2 factor is a logarithmic transformation of the sum-squared error of differences between the test and reference product over certain time points (Costa and Sousa Lobo, 2001). The ANMAT, like other regulatory agencies, considers that f_2 values ≥ 50 indicate an average difference of no more than 10% at the sample time points and ensure sameness or equivalence of the two compared curves (ANMAT, 2016). For selecting the last sample dissolution time point for the calculation of f_2 , the ANMAT criterion (ANMAT, 2016), namely “only one-time point should be considered after $\geq 85\%$ dissolution of the reference product,” was followed.

DE is defined as the area under the dissolution curve (AUC) up to a certain time, t , expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time (Costa and Sousa Lobo, 2001). DE was calculated from the (AUC, measured using the trapezoidal rule) at the last sample time point (40 minutes) and expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time.

MDT is the arithmetic mean value of any dissolution profile (Costa and Sousa Lobo, 2001; Podczeczek, 1993). To calculate the MDT, the AUC at 40 minutes (measured using the trapezoidal rule) was divided by the maximal amount of the active pharmaceutical ingredient (API) dissolved (Q_{max}) at the same time.

Results of DE and MDT corresponding to the different CLP tablet products were compared by a one-way analysis of variance (ANOVA). Then, as *post-hoc* procedures, pairwise comparisons of each test product against the reference were carried out by multiple comparisons using Dunnett's test.

Model-dependent methods

The model-dependent approach is also recommended for dissolution similarity testing (Stevens *et al.*, 2015). This relies on describing dissolution profiles through mathematical functions. Several kinetic models were used to compare the dissolution profiles of the different CLP tablet products tested, which were applied by considering the amounts of drug released from 7.5 to 40 minutes (products I–III and V–VIII as their profiles were non asymptotic) and from 7.5 to 20 minutes (product IV due to its asymptotic profile). The mathematical models used included

the zero-order and first-order kinetics, as well as the Hixson–Crowell, Higuchi, Weibull, Probit, and Gompertz models, and all are included in DDSolver, a free program for modeling and comparison of drug dissolution profiles (Zhang *et al.*, 2010). The adjusted coefficient of determination (R^2_{adj}), the Akaike information criterion (AIC), and the model selection criterion (MSC) were used to select the most appropriate model, and all are included in DDSolver (Uebbing *et al.*, 2017; Zhang *et al.*, 2010). The model which gave the highest R^2_{adj} , the lower AIC, and the largest MSC [an MSC value of more than two to three indicates a good fit (Zhang *et al.*, 2010)] was selected.

Powder X-ray diffraction

To identify the CLP form present in the tested dosage forms, tablets of each product were gently powdered using an agate mortar and pestle. Then, a top-loading polymethyl methacrylate specimen holder was carefully filled with a portion of powder sample. PXRD patterns were obtained at room temperature (20°C–25°C) on a D8 advance diffractometer (Bruker AXS, Karlsruhe, Germany) operated at 40 kV and 40 mA, with a CuK α source ($\lambda = 1.5418 \text{ \AA}$) between 2 and 40°2 θ with a step size of 0.05°2 θ and a scan rate of 6 seconds/step. The samples were rotated at 30 rpm during acquisition. Data were processed using the EVA software (Bruker AXS, Karlsruhe, Germany).

RESULTS AND DISCUSSION

Clopidogrel bisulfate containing products

Detailed information on the eight tablet products tested in this study is summarized in Table 1. All of these tablets were enteric-coated, except product II (Table 1), and had a declared dose (expressed as the clopidogrel base) of 75 mg. The average tablet weight exhibited differences between products, with five having average weights lower than 260 mg and another three being higher than 300 mg (Table 1). The percentage relative standard deviation (% RSD) for weight variation was found to be between 0.4 and 2.6, with no tablet differing in weight by more than double the established percentage for tablets and with the average weight ranging from 130 to 324 mg (i.e., $\pm 7.5\%$). Hence, all tablet products complied with the USP specification for weight variation (United States Pharmacopeia (USP), 2016b).

Regarding the indications and instructions for use provided on the secondary packaging and PILs, in particular concerning the storage conditions of CLP tablets, it was found that all products (except test II, which is available as unitary blisters without PIL) contained the same recommendations about the temperature and conditions of storing on boxes and patient leaflets, namely “store at room temperature below or up to 30°C” (Table A1). This finding is important since CLP is sensitive to both moisture and excessive heat (Lestari *et al.*, 2010) and also because of the indicated compliance with the Argentine regulations (ANMAT, 1996; Brevedan *et al.*, 2019) regarding the information on PILs and boxes. Moreover, this information is valuable for the possible interchangeability of the evaluated products, since the World Health Organization considers that the concept of interchangeability of multisource (generic) products includes the equivalence of the dosage form as well as of the indications and instructions for use (World Health Organization (WHO), 2017).

Table 1. Formulation compositions and selected physical properties of CLP tablets.

| Product | Excipients | Appearance | Weight (mg), ^a %RSD ^b |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| I (Film-coated tablet) | Hydrogenated castor oil, microcrystalline cellulose, hydroxypropyl cellulose, macrogol 6000, mannitol, pink opadry type 32K 14834 (lactose monohydrate, hypromellose, titanium dioxide, triacetin, red iron oxide), carnauba wax. | Pink, round, biconvex, engraved with «75» on one side and «1171» on the other side. | 256.3 (251.4 – 261.7), 1.2 |
| II | c | Off-white, round, biconvex, engraved with «SL» on one side and with indented line in center on the other side. | 248.2 (242.0–258.3), 2.1 |
| III (Film-coated tablet) | Lactose, microcrystalline cellulose, sodium croscarmellose, colloidal silicon dioxide, magnesium stearate, PEG 6000, propylene glycol, hydroxypropylmethylcellulose, talc, titanium dioxide, red iron oxide. | Brownish pink, round, biconvex, with indented line in centre on one side | 242.2 (239.3–244.4), 0.6 |
| IV (Film-coated tablet) | Anhydrous lactose, pregelatinised starch, microcrystalline cellulose, hydrogenated castor oil, hydroxypropylmethylcellulose, PEG 6000, titanium dioxide, red iron oxide, talc, magnesium stearate, triethyl citrate. | Dark pink, round, biconvex, engraved with «75» on one side. | 243.7 (237.3–256.3), 2.6 |
| V (Film-coated tablet) | Microcrystalline cellulose, sodium stearyl fumarate, colloidal silicon dioxide, crospovidone, anhydrous lactose, opadry II white, ponceau, opadry clear, saccharin sodium. | Fuchsia, round, biconvex | 306.8 (295.0–310.0), 1.4 |
| VI (Film-coated tablet) | Microcrystalline cellulose, sodium stearyl fumarate, colloidal silicon dioxide, crospovidone, anhydrous lactose, opadry II white, ponceau, opadry clear, saccharin sodium. | Fuchsia, round, biconvex | 308.9 (305.3–312.2), 0.7 |
| VII (Film-coated tablet) | Cellactose 80, sodium starch glycolate, talc, sodium stearyl fumarate, hydroxypropylmethylcellulose, PEG 6000, povidone, propylene glycol, titanium dioxide, colloidal silicon dioxide, yellow iron oxide, red iron oxide, brown iron oxide. | Brown, round, biconvex, engraved with «75» on one side | 241.2 (240.1–243.0), 0.4 |
| VIII (Film-coated tablet) | Anhydrous lactose, microcrystalline cellulose, pregelatinised starch, talc, colloidal silicon dioxide, simethicone, opadry, red iron oxide. | Pink, round, biconvex, with indented line in centre. | 299.7 (288.9–308.2), 1.9 |

^aAverage result for 12 dosage units followed by the range in parentheses.

^b% RSD.

^cProduct II was sold as unitary blisters without patient handout (hospital presentation).

Table 2. Dissolution test results for CLP products.

| Product | Stage S1 | Stage S2 |
|---------|----------------------------------------------------------|---------------------------------------------------------|
| | Percentage of label claim dissolved, ^{ab} % RSD | Percentage of label claim dissolved, ^{ab} %RSD |
| I | 96.8 (94.1–100.8), 3.0 | 93.1 (80.4–100.8), 6.9 |
| II | 85.3 (79.7–94.6), 6.9 | 85.3 (79.7–94.6), 5.2 |
| III | 92.4 (87.9–97.5), 3.6 | 96.3 (87.9–119.7), 8.8 |
| IV | 97.7 (94.9–103.3), 3.1 | 99.1 (94.9–105.6), 3.4 |
| V | 84.2 (74.2–89.7), 7.2 | 85.4 (70.8–97.8), 8.6 |
| VI | 89.0 (80.6–93.6), 5.3 | 88.5 (80.6–94.0), 5.1 |
| VII | 96.6 (93.2–99.2), 2.2 | 96.0 (90.7–102.1), 3.4 |
| VIII | 89.8 (83.2–97.5), 6.4 | 91.6 (83.2–102.5), 6.1 |

^aIn pH 2.0 HCl buffer. ^b Average result followed by their range in parentheses.

Dissolution test

Table 2 lists the results of the dissolution test in the pH 2.0 HCl buffer, while Figure 2 shows the mean dissolution profiles of the assayed products. The dissolution test described in USP 39 for CLP tablets indicates that not less than 80% (Q) of the labeled amount of API should be dissolved in 30 minutes (United States Pharmacopeia (USP), 2016a). Only products I, III, IV, and VII complied with the pharmacopeial specification in S1 stage (namely the first stage of dissolution test when six units are run, sampled, and analyzed for the dissolved amount of the active percentage) as the dissolved amount of each of the 6 units tested for those products was not less than Q + 5% (Table 2) in accordance with USP Acceptance Table 1 (United States Pharmacopeia (USP), 2016c). Thus, the other six tablets were analyzed in the second stage of the dissolution test, which is known as the S2 stage,

finding that all products fulfilled S2 dissolution criteria (namely the average of 12 units is equal to or greater than Q and no unit is less than Q–15%), with product III exhibiting the highest variability (%RSD = 8.8) (Table 2).

However, the dissolution profiles shown in Figure 2 indicate that the products had differences in dissolution performance, with the compendial medium providing good discrimination among the different formulations (Mayet *et al.*, 2008). Thus, statistical comparisons of the profiles were carried out.

In order to evaluate the similarity between test and reference dissolution profiles, the similarity factor (f_2) was calculated. As shown in Table 3, two f_2 values were greater than 50 (tests V and VI) and five f_2 values were lower than 50 (tests II–IV, VII, and VIII). Although the f_2 values for tests V and VI seemed to support dissolution profile similarity with Plavix[®] (f_2

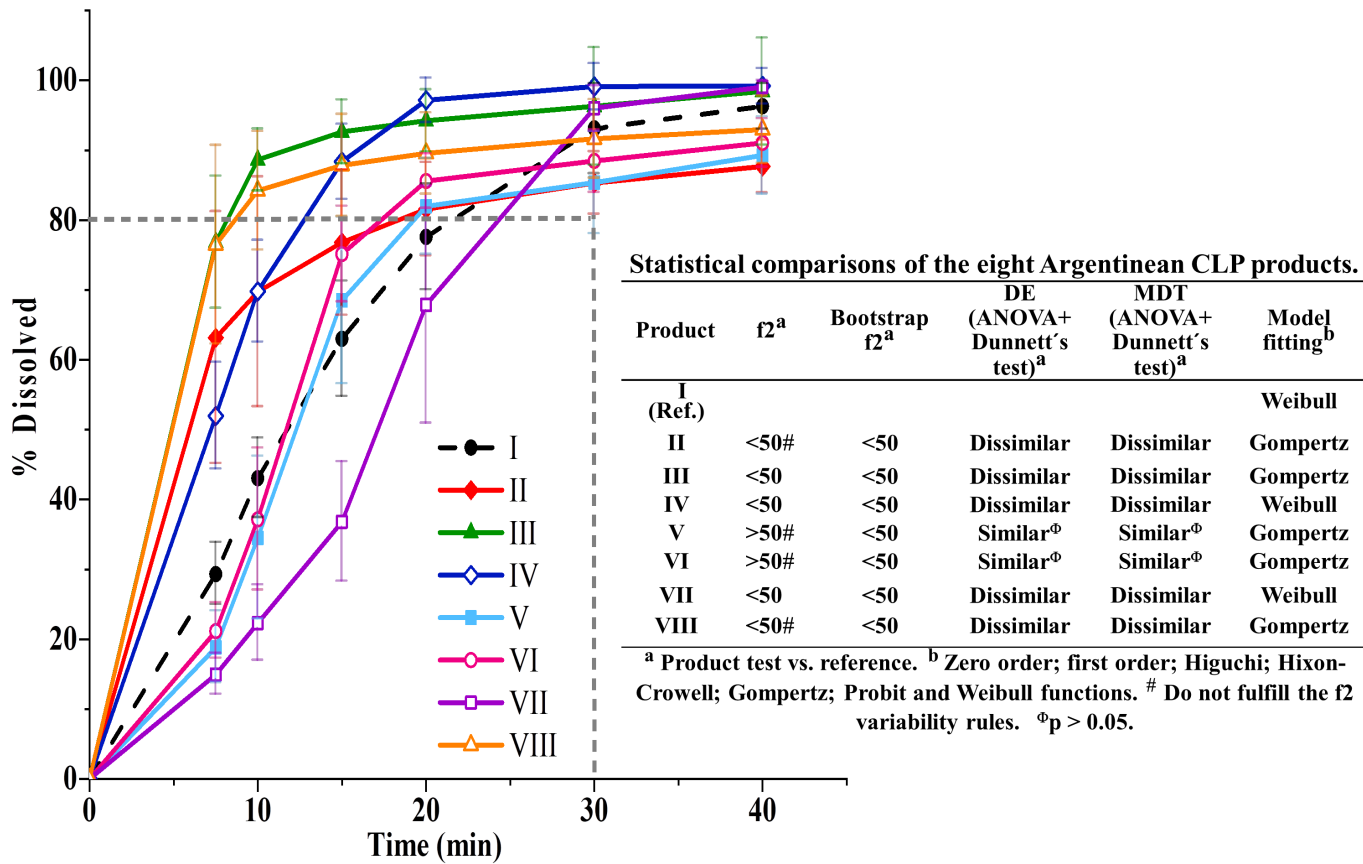


Figure 2. Dissolution profiles of CLP from products I–VIII in pH 2.0 HCl buffer. The intersection of the dashed lines represents the USP Q point. Each point represents the average percentage dissolved of 12 tablets. Bars indicated the standard deviation (\pm SD). Statistical comparisons for dissolution profiles showed conclusions of dissolution similarity between tested products and Plavix®.

> 50), the %RSD values at the earlier time points (<15 minutes) were >20% (ANMAT, 2016), indicating that these products, as well as tests II and VIII, did not fulfill the f_2 rules of variability. Because of this, the f_2 test could be used only for products III, IV, and VII, and their profiles were found to be nonsimilar ($f_2 < 50$) to that of product I (Table 3). Considering that the variability of four products exceeds the f_2 rules, other statistical methods were used, which included model-independent and model-dependent methods (Mendyk *et al.*, 2013; Paixão *et al.*, 2017; Shah *et al.*, 1998; Stevens *et al.*, 2015; Yoshida *et al.*, 2017). Among these approaches, the bootstrap (BS) methodology is recommended to assess the similarity of dissolution profiles having large variations (Mendyk *et al.*, 2013; Paixão *et al.*, 2017; Shah *et al.*, 1998; Stevens *et al.*, 2015; Yoshida *et al.*, 2017) since it allows for the use of f_2 , not as a point estimator but as a confidence interval, thus overcoming concerns encountered when f_2 is used solely as a point estimate (Stevens *et al.*, 2015). The method compares the lower limit of the 90% confidence interval of expected f_2 value (f_2^*) with the f_2 criterion to assess dissolution similarity (Yoshida *et al.*, 2017). The PhEq_bootstrap version 1.2 program (Mendyk *et al.*, 2013), an open source software available for the BS calculation, was used. Using the BS method, the seven test products could be declared dissimilar to Plavix® as all the f_2^* values were lower than 50 (Table 3).

Table 3. Similarity factor (f_2) and bootstrap-based f_2 (f_2^*) values for multisource CLP products available in Argentina.

| Products | f_2 | f_2^* | Conclusion ^b |
|----------|-------------------|---------|-------------------------|
| II / I | 34.2 ^a | 29.5 | Non-similar |
| III / I | 24.0 | 22.0 | Non-similar |
| IV / I | 33.5 | 30.3 | Non-similar |
| V / I | 55.3 ^a | 49.3 | Non-similar |
| VI / I | 54.0 ^a | 49.2 | Non-similar |
| VII / I | 38.6 | 34.1 | Non-similar |
| VIII / I | 25.7 ^a | 22.9 | Non-similar |

^aVariability exceeds the f_2 rules as the % RSD was > 20% at earlier sample dissolution time points.

^bBased on similarity criterion of bootstrap method: the lower confidence interval of the expected value of f_2 (f_2^*) is ≥ 50 (Kirk *et al.*, 2007; Paixão *et al.*, 2017).

Dissolution profiles were also compared using two model-independent approaches, namely DE (ANOVA, Dunnett's test) and MDT (ANOVA, Dunnett's test), with Figure 3 showing the average DE and MDT values for the assayed products.

The minimum and maximum DE and MDT values were found to be found to be 58.2% and 85.7% (DE) and 5.7 and 16.7 minutes (MDT), respectively, with an acceptable associated

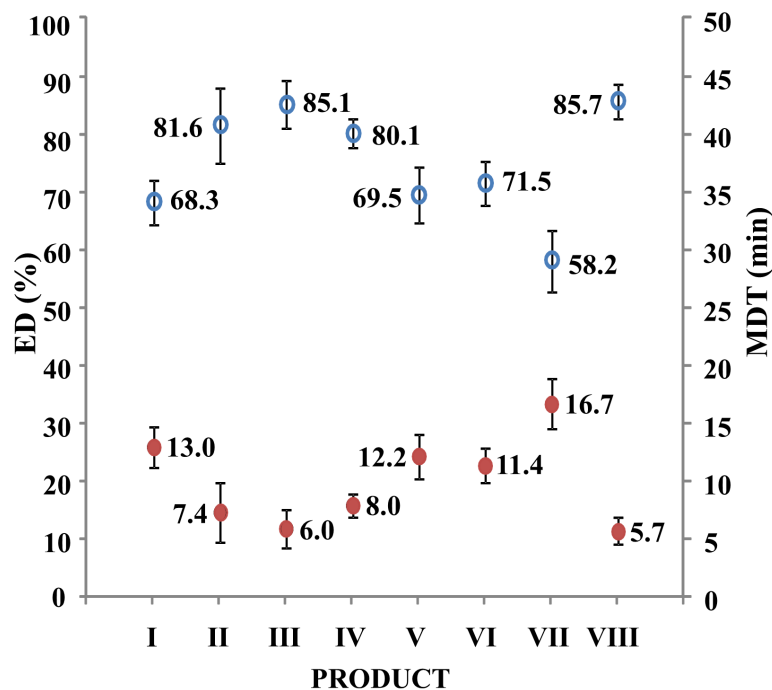


Figure 3. Comparison of DE and MDT values among the eight clopidogrel bisulfate products. Average values ($n = 12$) are indicated alongside the respective filled (MDT values) or unfilled circles (DE values). Bars indicate the standard deviation (\pm SD). (one-way ANOVA) for DE and MDT values.

One-way ANOVA for DE and MDT results.

| | Df ^a | F | P-value | Conclusion ^b |
|-----------|-----------------|--------|---------|-------------------------|
| DE media | 7 | 58.058 | 0.000 | Statistically different |
| MDT media | 7 | 56.615 | 0.000 | Statistically different |

^aDegrees of freedom. ^bPos hoc analyses are presented in Table A-3 and Table A-4.

variability in terms of %RSD (Table A2). Product VII showed the lowest DE and the highest MDT (16.7 minutes), indicating that its dissolution process was the slowest and most inefficient. Product VIII exhibited the lowest MDT (5.7 minutes) and highest DE values. However, when DE and MDT values of products II–VIII were compared with those of Plavix[®] using the Dunnett's test, products V and VI were found to be similar to brand I since no statistical differences ($p > 0.05$) were observed in the DE and MDT values (Tables A3 and A4).

The dissolution profiles were also evaluated by fitting the data to different mathematical models. Table 4 shows the values of the dissolution constants (k), model parameters, R^2_{adj} , AIC, and MSC values obtained by model fitting via DDSolver. The data presented in Table 4 show that the Gompertz and Weibull models (two nonlinear models which represent sigmoid functions) provide a very good fit ($R^2_{adj} \geq 0.992$) or a good fit ($R^2_{adj} = 0.848$ – 0.993) (Uebbing *et al.*, 2017), respectively, for the dissolution data of the tested products. These results agreed with those obtained by other authors (Costa and Sousa Lobo, 2001), who reported that both Weibull and Gompertz's functions frequently provide a good fit for different types of dissolution profiles. For brand I and products IV and VII, both R^2_{adj} values of the Weibull and Probit models revealed values close to unity, indicating that these two models can represent the dissolution of CLP. However, as the MSC values were slightly greater and the AIC ones slightly smaller for the Weibull model than for the Probit one, the Weibull model was selected. In the case of products II, III, V, VI, and VIII, both the R^2_{adj} and MSC values were greater and the AICs were smaller, when the data were adjusted to the Gompertz function, indicating that this model satisfactorily described the dissolution of CLP from these products. Accordingly, this model was chosen.

Figure A1 shows a graphical representation of the mathematical modeling of dissolution data for products I–VIII.

Since products IV and VII are fitted to the same dissolution rate model (Weibull function) as brand I, a statistical comparison of the profiles was performed by a t -test (two-sided) for the Weibull estimated parameters, with the medium α (scale factor) and β (shape factors which characterized the type of dissolution profile parameters being compared for the test products (IV and VII) against the reference (I). From these analyses, only the β parameter of product IV was found not to be significantly different from that of Plavix[®] ($t_{cal} < t_{crit}$), implying that the shape of its dissolution profile was similar to that of brand I. In contrast, the β parameter of product VII was found to be significantly different from that of the brand I ($t_{cal} > t_{crit}$), indicating that, in this case, the shape of its dissolution profile was different from that of the reference, despite both profiles being sigmoid (S -shaped with upward curvature, as both β values were > 1) (Costa and Sousa Lobo, 2001). Thus, although products IV and VII exhibited the same kinetic performance as brand I, they were revealed to be nonsimilar to Plavix[®] for one (scale factor α) and two (and β) Weibull parameters, respectively.

Identification of CLP polymorphic state in dosage forms

The identification of the polymorphic form present in dosage forms is important for quality control of products containing polymorphic APIs (Kassuha *et al.*, 2009; Koradia *et al.*, 2004), such as CLP, with PXRD being one of the analytical techniques available for this purpose (Klisuric *et al.*, 2013; Koradia *et al.*, 2004). Hence, tablets of products I–VIII were subjected to this analysis. Figure 4 shows the PXRD patterns obtained from powdered tablets of the tested products and also

Table 4. Parameters for the mathematical models and descriptive statistics for the dissolution data of the tested CLP products.

| Model | Statistics | Product | | | | | | | |
|----------------|------------|---------|---------|---------|--------|---------|---------|---------|---------|
| | | I | II | III | IV | V | VI | VII | VIII |
| Zero-order | k_0 | 2.984 | 3.054 | 3.524 | 5.576 | 2.830 | 2.945 | 2.769 | 3.350 |
| | R^2 adj | 0.612 | -11.878 | -30.213 | 0.565 | 0.554 | 0.449 | 0.919 | -47.800 |
| | MSC | 0.613 | -2.889 | -3.774 | 0.333 | 0.474 | 0.263 | 2.186 | -4.221 |
| | AIC | 45.557 | 53.958 | 56.913 | 27.077 | 47.419 | 48.814 | 39.819 | 56.560 |
| First-order | k_1 | 0.065 | 0.106 | 0.196 | 0.120 | 0.059 | 0.066 | 0.047 | 0.173 |
| | R^2 adj | 0.945 | 0.181 | 0.867 | 0.903 | 0.850 | 0.830 | 0.807 | -0.113 |
| | MSC | 2.576 | -0.133 | 1.686 | 1.837 | 1.563 | 1.438 | 1.312 | -0.440 |
| | AIC | 33.778 | 37.424 | 24.151 | 21.062 | 40.884 | 41.762 | 45.063 | 33.873 |
| Higuchi | kH | 15.727 | 16.961 | 19.761 | 21.710 | 14.894 | 15.588 | 13.957 | 18.827 |
| | R^2 adj | 0.905 | -1.537 | -6.743 | 0.938 | 0.762 | 0.742 | 0.728 | -11.595 |
| | MSC | 2.017 | -1.264 | -2.380 | 2.273 | 1.103 | 1.020 | 0.968 | -2.867 |
| | AIC | 37.131 | 44.210 | 48.549 | 19.315 | 43.644 | 44.270 | 47.125 | 48.434 |
| Hixson-Crowell | kHC | 0.018 | 0.029 | 0.037 | 0.032 | 0.017 | 0.019 | 0.014 | 0.035 |
| | R^2 adj | 0.982 | -0.501 | -0.951 | 0.977 | 0.871 | 0.857 | 0.869 | -2.973 |
| | MSC | 3.677 | -0.739 | -1.002 | 3.274 | 1.712 | 1.610 | 1.695 | -1.713 |
| | AIC | 27.171 | 41.061 | 40.279 | 15.311 | 39.990 | 40.729 | 42.763 | 41.510 |
| Weibull | α | 49.626 | 2.411 | 2.460 | 32.040 | 152.753 | 317.490 | 893.484 | 1.341 |
| | β | 1.436 | 0.454 | 0.672 | 1.573 | 1.842 | 2.192 | 2.295 | 0.364 |
| | T_d | 15.161 | 6.936 | 3.815 | 9.056 | 15.336 | 13.851 | 19.325 | 2.240 |
| | R^2 adj | 0.998 | 0.980 | 0.903 | 0.998 | 0.910 | 0.929 | 0.987 | 0.904 |
| | MSC | 5.783 | 3.480 | 1.888 | 5.651 | 1.960 | 2.196 | 3.916 | 1.901 |
| | AIC | 14.531 | 15.743 | 22.937 | 5.807 | 38.503 | 37.216 | 29.437 | 19.826 |
| | α | -3.344 | -0.668 | -1.011 | -3.410 | -4.043 | -4.507 | -5.343 | -0.143 |
| | β | 3.166 | 1.175 | 2.068 | 3.942 | 3.712 | 4.259 | 4.456 | 1.065 |
| Probit | R^2 adj | 0.997 | 0.990 | 0.925 | 0.996 | 0.950 | 0.952 | 0.962 | 0.921 |
| | MSC | 5.264 | 4.146 | 2.150 | 5.047 | 2.558 | 2.601 | 2.831 | 2.097 |
| | AIC | 17.647 | 11.750 | 21.367 | 8.221 | 34.914 | 34.786 | 35.949 | 18.654 |
| | α | 43.888 | 2.148 | 7.415 | 99.957 | 92.986 | 144.944 | 243.688 | 1.284 |
| Gompertz | β | 3.993 | 1.777 | 3.891 | 5.712 | 4.565 | 5.116 | 4.973 | 1.885 |
| | R^2 adj | 0.980 | 0.997 | 0.949 | 0.983 | 0.977 | 0.972 | 0.917 | 0.940 |
| | MSC | 3.470 | 5.292 | 2.542 | 3.475 | 3.324 | 3.121 | 2.042 | 2.377 |
| | AIC | 28.410 | 4.872 | 19.019 | 14.508 | 30.317 | 31.664 | 40.685 | 16.973 |

includes a comparison of the calculated patterns for polymorphs I (monoclinic form Chernyshev *et al.*, 2010) and II (orthorhombic form Bousquet *et al.*, 2003) of CLP, the two CLP polymorphs currently used in formulations, which were simulated using crystal structures obtained from the Cambridge Structural Database (Groom *et al.*, 2016) (version 5.38, updated to November 2016). Although the CLP tablets contained a large mass fraction of crystalline and amorphous excipients (Table 1), with the crystalline ones complicating the PXRD patterns of all the products and the amorphous excipients (e.g., microcrystalline cellulose) shifting the baseline, the identification of the characteristic reflections of CLP was possible in the eight products evaluated (Klisuric *et al.*, 2013; Koradia *et al.*, 2004). The reference brand and the remaining evaluated products, except product IV, exhibited the characteristic Bragg's peaks of polymorph II, namely at 8.8, 9.6, 12.3, 12.9, 13.0, 13.6, 18.5, 21.6, and 23.0°2 θ , indicating that products I-III and V-VIII have polymorphic equivalence.

Our result for Plavix® is in agreement with a previous survey (Pindelska *et al.*, 2015), which found that the product marketed in France contained the polymorph II, indicating consistency in the polymorphic state of the API in spite of the differences in manufacturing sites. In the case of product IV, the peaks related to form I (i.e., 9.2, 10.9, 11.5, 13.9, 14.4, 14.8, 20.6, 23.2, and 25.5°2 θ) were unambiguously identified, with no peaks due to form II being observed. In the case of other multisource products, formulations marketed in India (Koradia *et al.*, 2004) were found to contain form II, whereas generics commercialized in Serbia (Klisuric *et al.*, 2013) and Poland and Czech Republic (Pindelska *et al.*, 2015) contained polymorph I. It should be noted that, at present, the polymorphic inequivalence of CLP products is of no concern, evidently because both polymorphs have the same indications (Bousquet *et al.*, 2003), and products manufactured with different polymorphs (I or II) were found to be *in vivo* bioequivalent. In fact, in a bioequivalence study which compared

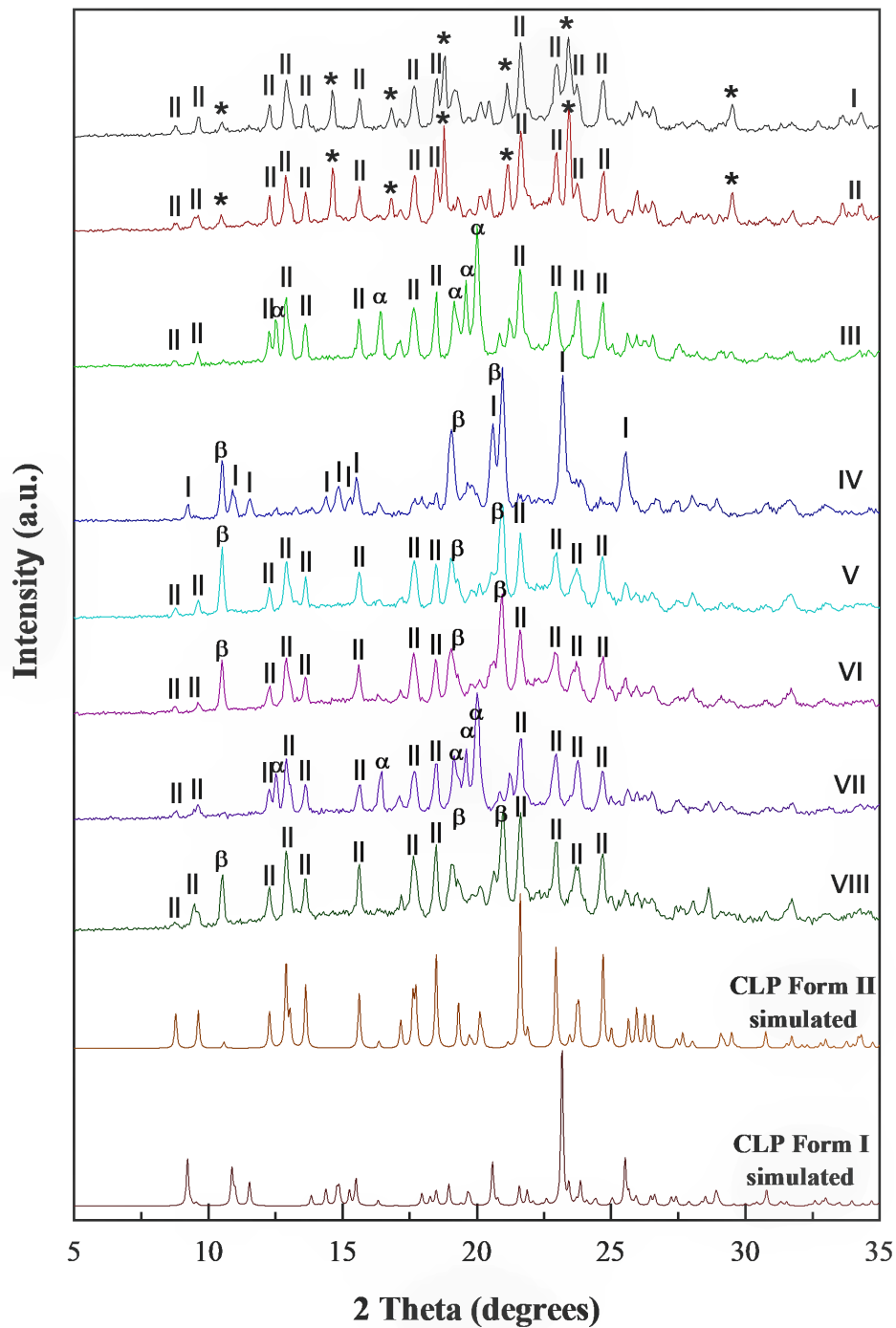


Figure 4. Experimental PXRD patterns of powdered tablets of products I–VII compared to the simulated patterns for CLP forms I and II. Key: I: CLP form I; II: CLP form II; *: mannitol; α: lactose monohydrate; and β: anhydrous lactose.

Plavix 75 mg tablets formulated with form I and with form II, the products demonstrated bioequivalence and equipotency (European Medicines Agency (EMA), 2004), and a generic CLP product (Zyllt 75 mg tablets) manufactured in Slovenia with polymorph I was considered bioequivalent to the originator Plavix® 75 mg tablets formulated with polymorph II (European Medicines Agency (EMA), 2009).

Using Figure 4, it was also possible to identify the main crystalline excipients of the tested products, with these results showing compliance with the formulas declared in the respective PILs (Table 1). For example, in the PXRD patterns of products I and II, the polymorph beta or I (Burger *et al.*, 2000) of mannitol (10.5, 14.6, 16.8, 18.8, 21.1, 23.4, and 29.5°2θ) can be easily identified. On the other hand, the powder patterns of products III and VII

exhibit the characteristic lines of α -lactose monohydrate (12.5, 16.4, 19.1, 19.6, and 20.0°2 θ) (Kirk *et al.*, 2007), whereas the patterns of products IV, V, VII, and VIII reveal the reflections of anhydrous β lactose (10.5, 19.0, 20.6, and 20.9°2 θ) (Kirk *et al.*, 2007).

Study limitations

In the present study, we assayed only one lot of the eight tested products mainly due to the economic and logistical difficulty of procuring samples of other lots. The evaluation of additional lots would be necessary to establish lot-to-lot reproducibility and to detect substandard lots, which is another important aspect of the quality control of multisource products. Furthermore, we only performed a quality control dissolution test (pH 2.0) according to USP 39, but as CLP is a BCS Class II API which shows limited solubility at intestinal pH (El-Laithy *et al.*, 2018), it would be important to evaluate the dissolution performance of the tested products in the three media used to establish similarity, that is, buffer solutions at pH 1.2, 4.5, and 6.8 (ANMAT, 2016; Paixão *et al.*, 2017; Stevens *et al.*, 2015), and even in biorelevant media such as fasted state simulated intestinal fluid (FaSSIF) and simplified “biorelevant” FaSSIF (Taupitz and Klein, 2010). These media could give a deeper evaluation of the dissolution performance of the tested products, with a view of predicting *in-vivo* absorption and bioavailability differences. The assessment of the dissolution behavior of additional lots in the USP medium and the evaluation of the similarity of the seven multisource products with Plavix® in biorelevant media, which can simulate *in vitro* various conditions that are found *in vivo*, are intended future undertakings.

CONCLUSION

This is the first study that compared the dissolution performance of several CLP multisource products available in Argentina with the innovator brand (Plavix®). The evaluated products were found to comply with the requirements of the dissolution test of the USP 39 and were considered pharmaceutical equivalents to Plavix®. However, the dissolution profiles of the tested products were not superimposable to that of Plavix®. Indeed, only products V and VI were similar to Plavix® in terms of DE and MDT values ($p > 0.05$). Kinetically, only products IV and VII are fit the same dissolution rate model (Weibull function) as Plavix®, but they showed differences in the Weibull parameters. It was also observed that six multisource products contained polymorph II, as in the case of Plavix®, with one (product IV) having form I. However, the polymorphic inequivalence of this product is of no concern since polymorphs I and II have the same indications and, currently, there are no regulatory specifications with regard to which polymorph has to be formulated in CLP 75 mg tablets. Our results also indicated that the USP method could be convenient (Mayet *et al.*, 2008) for evaluating the dissolution performance of CLP products. This is an important finding from the biopharmaceutical quality control point of view, since USP Apparatus II (paddle apparatus) is available in many quality control laboratories, and the HCl buffer is a cheap dissolution medium. However, *in vivo* bioequivalence studies should be conducted to try to confirm any correlations with the *in vitro* performance of the CLP multisource products tested and to demonstrate their interchangeability with the innovator brand since none of them have been recognized by the ANMAT as bioequivalent to Plavix®.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interest.

AUTHORS' CONTRIBUTION

All the authors have made substantial contributions to the work and have read and approved the final manuscript.

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