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Influence of Storage Conditions on Pharmaceutical Equivalence and Similarity of Hydrochlorothiazide Tablets in Argentina

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ABSTRACT

Similarity studies are used with the intention to establish interchangeability of certain formulations in vitro, without the need to carry out in vivo studies. This interchangeability between formulations should be conserved during the product shelf life, as an integral part of the pharmaceutical stability. Hydrochlorothiazide (HCTZ), a widely used diuretic, is classified as a class 3 drug in the Biopharmaceutics Classification System (BCS). An immediate-release (IR) solid oral formulation containing a class 3 drug is a candidate for biowaiver if it meets the requirement of ˙very rapid dissolution' (i.e., ≥ 85% dissolved within 15 minutes). This research aimed to compare four solid oral IR HCTZ formulations (50 mg), commercially available in Argentina, with respect to pharmaceutical similarity and stability. To assess for similarity, dissolution profiles were compared both at time zero (T0) and after 12 months of storage (T12) at pH 1.2, 4.5, and 6.8. To assess for stability, critical quality attributes (CQAs) of the samples were evaluated at T12 of storage (25 °C, 60% relative humidity). At T12, all samples met the requirements for CQAs (i.e., assay, friability, disintegration, uniformity, and dissolution). The reference formulation had the fastest dissolution rate, and sample D was the slowest. Two multisource formulations exhibited statistical differences with respect to the reference sample, both at T0 and T12. Because the evaluated multisource formulations did not meet criteria for very rapid dissolution, they are not candidates for biowaivers. In terms of stability, some statistical differences were detected between dissolution performance at T0 and T12 (for some samples and dissolution media), but no specific pattern could be detected. Thus, it can be considered that the storage conditions did not affect the critical quality attributes of evaluated samples, and their pharmaceutical equivalence was maintained.

Keywords: Storage, biowaiver, critical quality attributes, hydrochlorothiazide (HCTZ), stability, dissolution

INTRODUCTION

In vitro dissolution evaluation under biowaiver conditions (i.e., similarity studies) is used to establish interchangeability of pharmaceutical products. The implementation of similarity studies avoids the requirement of in vivo evaluation, which is more complex, expensive, and with ethical implications. In this framework, the Biopharmaceutics Classification System (BCS) is used to identify compounds that may be eligible for similarity studies. Under biowaiver conditions, in vivo proof of bioequivalence may be replaced by in vitro dissolution studies, comparing test and reference products (*1*–*3*). This BCS-based biowaiver scheme presents important advantages such as cost savings,

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time savings, and avoidance of unnecessary testing in humans (*3*, *4*).

Currently, the American and European Agencies (FDA and EMA, respectively) and the World Health Organization (WHO) grant BCS-based biowaivers for products containing class 1 or 3 drugs (*1*, *5*, *6*). The same concepts are considered by the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use, and Argentina adopted these international guidelines and published their own local documents (*7*–*9*).

Hydrochlorothiazide (HCTZ) is a thiazide diuretic that blocks the specific sodium transport in the nephron; it is mainly used for the treatment of arterial hypertension and congestive heart failure at doses of 25 and 50 mg in solid oral dosage form (*10*, *11*). There is not agreement in the classification criterion of HCTZ, according to the BCS. However, most authors classify it as a class 3 drug, with high solubility and low permeability (*12*–*15*).

For class 3 drugs, the BCS-based biowaivers scheme can be applied if the test and reference products meet criteria for very rapid dissolution (VRD, i.e., ≥ 85% of labelled amount of drug dissolves within 15 minutes) in three buffer solutions (pH 1.2, 4.5, and 6.8), and if the excipients are qualitatively the same and quantitatively similar between formulations (*1*, *5*–*9*, *16*, *17*).

In Argentina, HCTZ tablets are available both as reference and multisource products, and patients commonly use them interchangeably. In prior work, four solid oral immediate release (IR) HCTZ formulations commercially available in Argentina were evaluated in terms of critical quality attributes (CQAs, i.e., evaluation of labels and patient information leaflets, description and mean weight of tablets, uniformity of dosage units, assay, hardness, friability, disintegration, and dissolution) (*18*). Similarity studies between reference and multisource formulations were not performed at that time.

Additionally, another critical parameter to be considered is pharmaceutical stability, which could be defined as the evidence provided on how the quality of a drug substance or drug product varies with time, under the influence of a variety of environmental factors (e.g., temperature and relative humidity) (*19*). During storage, a drug product may undergo changes in CQAs; in particular, when considering dissolution stability as a parameter, the bioavailability of the drug can be affected (*20*).

For these reasons, this research aimed to assess similarity (in terms of in vitro dissolution under biorelevant conditions) and pharmaceutical stability (in terms of CQAs) of four IR HCTZ formulations, after 12 months of storage at 25 °C with 60% relative humidity.

MATERIALS AND METHODS

Materials

HCTZ was purchased from Saporiti (Parafarm, Argentina). Analytical grade reagents (Anedra, Argentina) and distilled water were used for the assay and in vitro dissolution test. Three buffer solutions (pH 1.2 hydrochloric acid [HCl], pH 4.5 acetate, and pH 6.8 phosphate) prepared according to USP were used as dissolution media under biowaiver conditions (*21*).

HCTZ (50 mg) tablets were acquired in the local pharmaceutical market, and randomly labeled from A to D, with sample B the reference formulation. The excipient composition of these samples has been reported in the previous study (*18*).

Critical Quality Attributes Evaluation

For weight variation determination, 10 tablets of each sample were separately weighed (Acculab ALC-210.4M). Results were expressed as mean weight and standard deviation (SD).

Friability, hardness, and disintegration tests were performed according to the *Argentine Pharmacopeia* (22). For the first test, 10 tablets from each commercial sample were weighed and placed in the friability tester (Scout FGMO2). After 100 revolutions, the tablets were removed and weighed, expressing the weight loss as a percentage. For hardness determination, 10 individual tablets of each sample were analyzed (Scout DGM02), displaying the results as the degree of force in kilopounds (kp) required to break each dosage unit. Finally, a disintegration tester (Scout EGMO2) was used to measure the disintegration time of six tablets of each sample, in water at 37.0 ± 2.0 °C. The time required for the complete disintegration of each dosage unit was recorded, with a maximum analysis period of 30 minutes.

Assay and uniformity of dosage unit tests were performed by the application of UV-spectrophotometry (Varian Cary 50 Conc) at 272 nm (*22*). For the assay, an accurately weighed portion of the powdered tablets (n = 20) was dissolved in 0.1-M sodium hydroxide and diluted with water. For uniformity of dosage units, 10 individual tablets were similarly treated. In both cases, a standard calibration curve ($y = 0.0530x - 0.0133$; $R^2 = 0.9998$) was applied for the measurement of HCTZ concentration.

For dissolution tests, a USP apparatus 1 (Erweka DT60) at 100 rpm was utilized (*22*). The dissolution media consisted of 900 mL of 0.1-M HCl, and the sampling time was at 60 minutes (*22*). For sample filtration, 0.45-µm nylon membrane filters (Microclar) were used. The quantification of dissolved HCTZ was achieved by the application of a standard calibration curve ($y = 0.0650x - 0.0028$; $R^2 = 0.9996$; concentration range 2.0–12.0 µg/mL) at 272 nm. The specification for this test indicates that not less than 60% (Q) of the labeled amount of HCTZ should dissolve within the sampling time (*22*).

Dissolution Profiles Under Biowaiver Conditions

For dissolution profiles, the same equipment and agitation speed used for dissolution test were applied, with 900 mL of pH 1.2, 4.5, and 6.8 buffer solutions as dissolution media (*1*, *5*, *7*, *9*). Samples were collected at 5, 10, 15, 30, 45, 60, and 75 minutes, and filtered as stated in the previous section. UV-spectrophotometry, at 272 nm, was also applied for the quantification of HCTZ dissolved, with specific calibration curves for each media (concentration range 2.5–13.0 µg/mL, R^2 between 0.9996 and 0.9998). For statistical comparison purposes, analysis of variance (ANOVA) was applied for dissolution efficiency (DE) results. This parameter represents the ratio of the area under the curve of a particular dissolution profile with respect to the total area of a rectangle calculated considering the 100% dissolved as width, and the same time interval as length (*23*).

Pharmaceutical Stability

A pharmaceutical stability chamber (SCT Pharma), calibrated at 25 °C and 60% relative humidity (i.e., ICH natural conditions for climatic zone II) was used (*19*). After 12 months of storage of the samples (T12) in their original packaging, all CQA were evaluated, and statistically compared (ANOVA) with the results obtained at time zero (T0), which were published previously (*18*). Dissolution profiles under biorelevant conditions were

statistically compared in terms of DE and similarity.

RESULTS AND DISCUSSION

Critical Quality Attributes

The results of quality control tests at T12 are shown in Table 1 and Figure 1.

Tablet mean weights were 150.1–251.3 mg, with statistical differences detected between samples. These variable results could be explained based on differences in composition of evaluated samples at T0, reported previously (*18*). All evaluated samples complied with the specifications for hardness and disintegration tests, with mean values of 3.3–9.9 Kp and complete disintegration before 30 minutes (0.3–9.9 min). The assay specification for HCTZ tablets states that the acceptable range is 90.0–110.0% of the labeled amount (*21*, *22*). Assay results ranged from 96.3% ± 2.9 (sample D) to 101.6% ± 3.5 (sample A), therefore all samples met the requirements. The requirements for uniformity of dosage unit test (i.e., drug content is 85.0–115.0% of the labeled amount, relative standard deviation < 6.0%) and dissolution test in Stage 1 were also satisfied.

Figure 1 shows the dissolution profiles of the evaluated products in 0.1-N HCl. The reference (sample B) exceeded 60% dissolved after 5 minutes of starting the test, whereas sample D reached 60% dissolved after 10 minutes. Samples A and B fulfilled the definition of very rapid dissolution (VRD i.e., ≥ 85% dissolved within 15 minutes), and sample C had rapid dissolution (RD, i.e., ≥ 85% dissolved within 30 minutes).

Statistical comparison of HCTZ dissolution profiles was performed in terms of DE results, with mean values ranging from 69.1% to 89.0%. There are highly significant differences ($p < 0.01$) between D and the other samples and significant differences ($p < 0.05$) between samples B and C.

In summary, all the tested samples fulfilled the requirements for the evaluated CQAs at T12 (and at T0 as previously reported) (*18*).

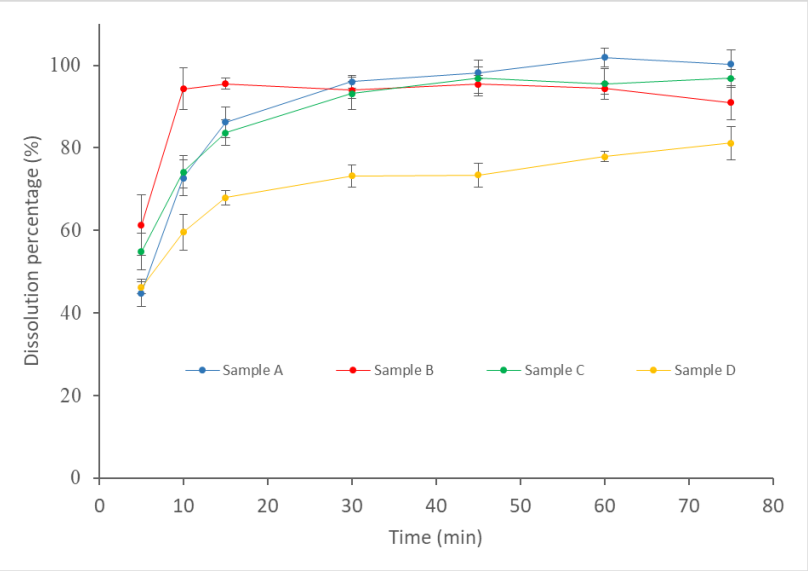


Figure 1. In vitro dissolution profile in 0.1 M hydrochloric acid (HCl) at T12. Results presented as mean percentage of labeled amount dissolved ± SD. Sample B is the reference formulation.

Table 1. *Evaluation of Critical Quality Attributes at T12.*

Sample	Tablet weight (mg)	Hardness (Kp)	Friability (% weight loss)	Disintegration Time (s)	Assay (% labeled amount)	Uniformity (% labeled amount / RSD)	Dissolution, S1 Stage (% labeled amount dissolved / RSD)	DE (%)
A	150.1 ± 6.5	3.3 ± 0.2	0.72	356	101.6 ± 3.5	98.6–107.2 / 3.4	98–105 / 2.3	88.6 ± 1.5
B (Ref.)	199.4 ± 2.1	9.9 ± 0.5	0.25	188	94.6 ± 1.2	93.4–96.4 / 1.3	94–96 / 1.3	89.0 ± 1.6
C	251.3 ± 2.0	7.3 ± 0.6	0.29	19	97.0 ± 1.7	95.2–99.0 / 1.8	89–99 / 3.7	86.6 ± 2.9
D	160.7 ± 2.4	5.5 ± 0.4	0.18	80	96.3 ± 2.9	92.4–98.4 / 3.0	77–79 / 1.2	69.1 ± 1.6

Values are mean ± SD or range unless otherwise noted.

Ref: reference formulation; RSD: relative standard deviation; DE: dissolution efficiency

Table 2. Dissolution Efficiency (DE) Results at T0 and T12.

Sample		A			B (Ref.)		C			D		
Buffer Solution and Time Point		DE	SA		DE	SA	DE	SA		DE	SA	
			I[a]	II[b]		II[b]		I[a]	II[b]		I[a]	II[b]
pH 1.2	T0	82.5 ± 1.8 (RD)	ns	**	83.5 ± 0.8 (VRD)	**	81.4 ± 1.3 (RD)	**	*	69.9 ± 1.4	**	ns
	T12	91.5 ± 2.4 (RD)	ns		92.4 ± 1.0 (VRD)		86.0 ± 2.3 (RD)	**		71.2 ± 1.6	**	
pH 4.5	T0	85.9 ± 6.4 (RD)	ns	ns	89.5 ± 1.4 (VRD)	ns	75.6 ± 8.1	**	ns	68.1 ± 2.7	**	*
	T12	85.9 ± 0.6 (RD)	*		90.7 ± 0.9 (VRD)		72.0 ± 6.0	**		63.7 ± 2.8	**	
pH 6.8	T0	93.0 ± 1.4 (VRD)	**	ns	95.3 ± 1.3 (VRD)	**	75.4 ± 5.1	**	**	66.9 ± 1.2	**	**
	T12	91.1 ± 4.2 (VRD)	ns		91.8 ± 1.9 (VRD)		69.8 ± 3.1	**		59.2 ± 1.3	**	

Results are expressed in % of labelled amount dissolved (mean ± SD)

[a]Statistical comparison between DE values of multisource formulation and reference sample B, at each storage time and dissolution medium.

[b]Statistical comparison between DE values at T0 and T12, for every single sample, at each dissolution medium.

**p < 0.05; **p < 0.01*

Ref: reference formulation; SA: statistical analysis; RD: rapid dissolution (i.e., ≥ 85% dissolved within 30 minutes); VRD: very rapid dissolution (i.e., ≥ 85% dissolved within 15 minutes); ns: no significant differences.

Dissolution Profiles

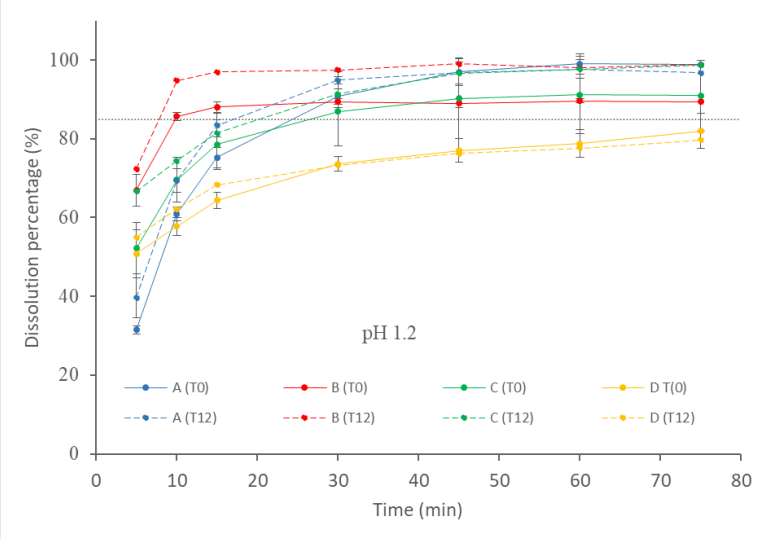
Dissolution profile data are presented in Table 2 and Figure 2.

At pH 1.2 T0, the lowest dissolution performance of sample D stands out. The reference (sample B) fulfilled the VRD definition, whereas samples A and C fulfilled the RD definition. At 60 minutes, samples A, B, and C reached 91–99% dissolution while sample D did not reach 80%. Highly significant differences were detected in the comparison of DE between B, C, and D. At T12, the samples retained their dissolution profile in shape and rate. At 60 minutes, samples A, B, and C dissolved 20% more HCTZ than sample D. Highly significant differences were detected between samples B, C, and D in terms of DE.

At pH 4.5 T0, the dissolution performance of samples A, B, and D were maintained as in pH 1.2, although sample C did not fulfill the criteria for RD. At 60 minutes, samples A, B, and C dissolved approximately 15% more HCTZ than sample D. Highly significant differences in DE was detected between samples B, C, and D, while there were no significant differences with sample A. At T12, a small variation could be observed in the dissolution profiles of samples A and B, while a decrease was recorded for C and D. In this case, the statistical evaluation of DE yielded almost the same results as T0, although significant differences were observed between B and A.

At pH 6.8 T0, the lowest dissolution performance was observed for samples C and D, while formulations A and B had VRD profiles. Highly significant differences were detected between the reference and all multisource formulations in terms of DE. At T12, these differences were maintained for all samples, including a highly significant decrease in dissolution of HCTZ in samples C and D.

In summary, the reference product showed the fastest dissolution rate and fulfilled the VRD criterion in all three media and both storage conditions (Fig. 2). Under the same conditions, samples C and D showed highly significant differences with respect to the reference and the slowest dissolution rate. In fact, sample D did not reach 85% dissolution even after 75 minutes of analysis. Statistical differences in dissolution may be unrelated to formulation differences because all samples were similar based on CQA evaluation at T0 and T12. However, under the BCS-based biowaiver scenario, the multisource samples would not be considered similar to the reference due to differences in dissolution performance (i.e., slower rate and efficiency).



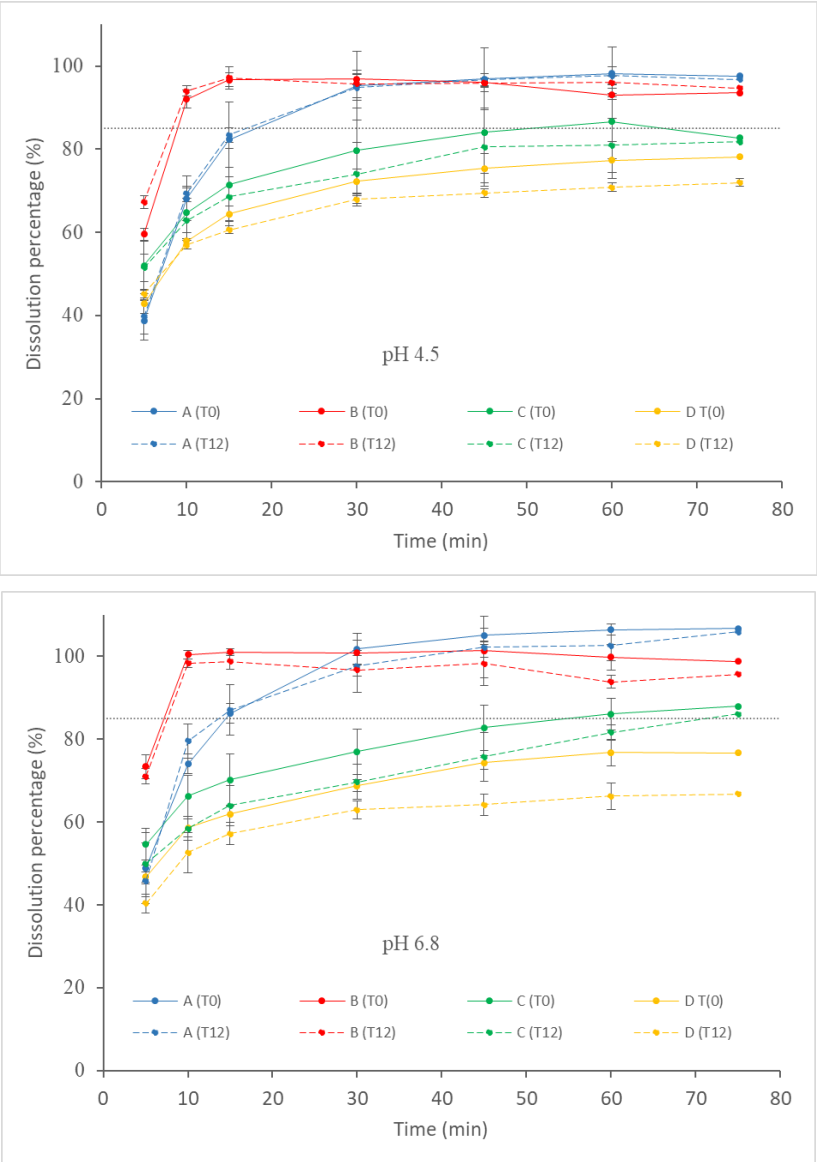


Figure 2. In vitro dissolution profiles under biowaiver conditions at T0 and T12. Results are mean percentage of labeled amount dissolved ± SD. Dotted black line indicates the '85% dissolved' level for RD and VRD definitions. Sample B is the reference formulation.
RD: rapid dissolution; VRD: very rapid dissolution.

CONCLUSION

All evaluated formulations could be considered pharmaceutical equivalents after 12 months of storage on the basis of CQA evaluation (i.e., tablet mechanical properties, assay, disintegration time, and dissolution test). In a biowaiver scenario, the multisource formulations could not be considered similar to the reference (at T0 at T12), because they did not fulfill the VRD requirement for BCS class 3 drugs.

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CONFLICTS OF INTEREST

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

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