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Original Article

In vitro biopharmaceutical equivalence of drugs used in the treatment of tuberculosis in Peru

[Equivalencia biofarmacéutica *in vitro* de medicamentos utilizados en el tratamiento de la tuberculosis en el Perú]

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Abstract

Context: Medicines are vital for public health, and it is essential to ensure their quality. This is especially important with multisource medications, which have not undergone clinical trials and may have limited bioavailability.

Aims: To determine the *in vitro* biopharmaceutical equivalence of drugs used in the treatment of tuberculosis in Peru.

Methods: The study evaluated multiple products for rifampicin, isoniazid, and pyrazinamide. Different dissolution conditions and sampling times were used. Similarity was determined following WHO guidelines.

Results: The *in vitro* biopharmaceutical characteristics (dissolution profiles) of the two multisource rifampicin products were different, with similarity factors (f2) less than 50 in all three dissolution media. For isoniazid, the profiles were similar, with more than 85% dissolution at 15 minutes in all media. For pyrazinamide, two profiles were similar, and one was different, showing less than 85% dissolution in all three media.

Conclusions: Multisource rifampicin products did not prove to be *in vitro* biopharmaceutical equivalence to each other, while isoniazid products were. Furthermore, in the case of pyrazinamide, two products were found to be equivalent, while one was not.

Keywords: antitubercular agents; biological availability; drug liberation; therapeutic equivalency.

Resumen

Contexto: Los medicamentos son vitales para la salud pública y es esencial asegurar su calidad. Esto es especialmente importante con los medicamentos multifuente, que no han sido sometidos a ensayos clínicos y pueden tener una biodisponibilidad limitada.

Objetivos: Determinar la equivalencia biofarmacéutica *in vitro* de medicamentos utilizados en el tratamiento de la tuberculosis en el Perú.

Métodos: En el estudio se evaluaron múltiples productos para rifampicina, isoniazida y pirazinamida. Se utilizaron diferentes condiciones de disolución y tiempos de muestreo. La similitud se determinó siguiendo las pautas de la OMS.

Resultados: Las características biofarmacéuticas *in vitro* (perfiles de disolución) de los dos productos de rifampicina de múltiples fuentes fueron diferentes, con factores de similitud (f2) menores de 50 en los tres medios de disolución. En el caso de la isoniazida, los perfiles fueron similares, con más del 85 % de disolución a los 15 minutos en todos los medios. En el caso de la pirazinamida, dos perfiles fueron similares y uno fue diferente, mostrando menos del 85 % de disolución en los tres medios.

Conclusiones: Los productos de rifampicina multifuente no demostraron ser equivalentes biofarmacéuticamente entre sí *in vitro*, mientras que los productos de isoniazida sí lo fueron. Además, en el caso de la pirazinamida, se encontró que dos productos eran equivalentes, mientras que uno no lo era.

Palabras Clave: agentes antituberculosos; biodisponibilidad; disolución; equivalencia terapéutica.

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INTRODUCTION

Medicines are essential elements in the public health of countries, so their quality must be ensured to avoid therapeutic failures or other unwanted effects (OMS, 2020). The characteristics of the medications that are dispensed must be investigated, especially the bioavailability characteristics of so-called multisource pharmaceutical products, since these have not been subject to clinical trials (FDA, 2021; Mendoza-Chuctaya et al., 2019; Saal and Nair, 2020)

The bioavailability of drugs is influenced by the technology used in the manufacturing processes. Factors such as the dosage form, whether solid (uncoated tablets, coated tablets, hard capsules, soft capsules) or liquid (suspensions), as well as the crystalline form of the active ingredient (API) and the nature and amount of excipients in the formulation, can significantly affect bioavailability (ICH, 2019; Molavi et al., 2020).

To have access to effective, safe, and high-quality drugs, it is necessary that those that are pharmaceutical equivalents are also therapeutic equivalents in such a way that they can be interchangeable between them (MINSA, 2018a).

Therapeutic equivalence between drugs is evaluated by standardized *in vivo* and *in vitro* methods; *in vitro* studies are prioritized. Government institutions related to the marketing of drugs in the world (DIGEMID, WHO, FDA, ICH, EMA) have considered exempting drugs from *in vivo* studies, which, according to the biopharmaceutical drug classification system (BCS), are class I (high solubility – high permeability) and class II (high solubility – low permeability). However, it is important to continue researching drugs from classes II (low solubility – high permeability) and class IV (low solubility – low permeability), with the aim of incorporating knowledge to promote biowaiver (Bransford et al., 2020; Davit et al., 2016; MINSA, 2018a; USP, 2019; WHO, 2017). These studies have a scientific basis related to the physicochemical characteristics, specifically solubility in aqueous media at physiological pH 1.2, 4.5, and 6.8 (Amidon et al., 1995; Saal and Nair, 2020; WHO, 2017). National and international directives have established that the test for evaluating therapeutic equivalence using dissolution profiles is the similarity factor - *f2* (Abend et al., 2023; FDA, 2017; MINSA, 2018a; USP, 2019; WHO, 2017).

Tuberculosis (TBC) is an infection caused by bacteria (*Mycobacterium tuberculosis* or Koch bacillus) that affects various organs such as bones, meninges, and kidneys, but most frequently the lungs (Muñoz del Carpio-Toia, 2018). TBC is transmitted from person to person by air through the inhalation of droplets containing these bacilli that are expelled through coughing and sneezing; even a brief inhalation of these droplets can be enough to cause an infection (WHO, 2023a).

The world population is infected with *Mycobacterium tuberculosis*, approximately a quarter. Bacterial resistance to anti-tuberculosis drugs is a concern, and the cost of tuberculosis treatment is very high. Globally, the number of drug-resistant patients from 2018 to 2022 was 1.5 million. In addition, it is known that the expense cost of this treatment fell from 5.8 billion to 5.3 billion dollars, less than half of what is needed (WHO, 2023b).

The quality of anti-tuberculosis drugs has an impact on the treatment of TBC. It is very important to ensure their quality. In Peru, medicines are acquired through tenders, where purchases are made from different laboratories. As a result, it cannot always be guaranteed that these medicines are bioequivalent, therapeutic equivalent or interchangeable with each other (MINSA, 2018a; 2018b).

The anti-tuberculosis drugs prescribed in Peru, among others, are rifampicin (300 mg capsules), isoniazid (100 mg tablets) and pyrazinamide (500 mg tablets). A failure in its quality can cause serious health problems, both from a therapeutic point of view and from its bacterial resistance (MINSA, 2018b; Quispe et al., 2020). The primary regimen for the treatment of tuberculosis, called RIPE, consists of rifampicin, isoniazid, pyrazinamide, and ethambutol. The first three are the most important and powerful because of their great bactericidal activity. When resistance to these drugs is generated individually or in combination, they cause monoresistant tuberculosis, polyresistant tuberculosis, and, most worryingly, multidrug-resistant tuberculosis, which has a very detrimental impact on public health (Quispe et al., 2020).

Strauch et al. investigated whether the *in vitro* procedure called "biowaiver" can be applied to anti-tuberculosis drugs. To do this, they tested bioequivalence procedures using pharmacokinetic parameters and the biowaiver procedure (comparison of dissolution profiles in three dissolution media at pH 1.2, 4.5, and 6.8. They concluded that the *in vitro* procedure guaranteed the therapeutic equivalence of isoniazid, pyrazinamide, and ethambutol tablets, establishing that the dissolution profiles at physiological pHs correlate with the biopharmaceutical characteristics (Strauch, 2011).

The aim of this research is to determine the *in vitro* biopharmaceutical equivalence of drugs used in the treatment of tuberculosis in Peru.

MATERIAL AND METHODS

Materials and reagents

Pharmaceutical products: The rifampicin capsules (300 mg), isoniazid tablets (100 mg), and pyrazinamide tablets (500 mg) were obtained through donations requested by the Center of Excellence for Tuberculosis Management of the Regional Docente de Trujillo (CENEX TB HRDT) - La Libertad – Perú.

The standards used were from the Sigma-Aldrich brand, USA.

Reagents: 37% hydrochloric acid (EMSURE® - Germany), 99.7% glacial acetic acid (J.T. Baker, USA), hydroxide sodium (EMPLURA® - Germany), 99.65% anhydrous sodium acetate (J.T. Baker, USA) and 99.63% monobasic potassium phosphate (J.T. Baker, USA). All reagents were of American Chemical Society (ACS) grade.

Placebo: Various pharmaceutical products from multisources were evaluated: two formulations of rifampicin 300 mg capsules (RA and RB), two formulations of isoniazid 100 mg (IA and IB), and three formulations of pyrazinamide 500 mg (PA, PB, and PC). Inactive ingredients contained in the products were: 1) rifampicin RA capsules (microcrystalline cellulose, povidone, starch, sodium lauryl sulfate, purified talc, magnesium stearate, hard gelatin capsule - carmoisine, sunset yellow, ponceau, titanium dioxide, gelatin, sodium methylparaben, sodium propylparaben, lauryl sulfate), RB (lactose monohydrate, magnesium stearate, sodium starch glycolate); isoniazid IA tablets (dibasic calcium phosphate, corn starch, edetate disodium, purified talc, colloidal anhydrous silica, magnesium stearate); 2) isoniazid IB tablets (lactose monohydrate, povidone, microcrystalline cellulose, hypromellose, corn starch, magnesium stearate, colloidal silicon dioxide) and 3) pyrazinamide tablets PA (croscarmellose sodium, microcrystalline cellulose, povidone, talc, magnesium stearate, alcohol, and purified water), PB (purified water, corn starch, sodium starch glycolate, magnesium stearate, mannitol, povidone, and colloidal anhydrous silica) and PC (corn starch, povidone, colloidal anhydrous silica, sodium starch glycolate, purified talc, magnesium stearate, and purified water).

Preparation of dissolution media

The dissolution media pH 1.2, 4.5, and 6.8 were prepared following the procedures described in USP 42 (USP, 2019).

Quantification of rifampicin

To quantify temporarily dissolved rifampicin, a spectrophotometric analytical methodology was designed and validated in the visible range for each dissolution media (pH 1.2, 4.5, and 6.8). The wavelength of maximum absorbance for the three media was 475 nm.

A calibration curve was prepared from a solution of the secondary standard of rifampicin in methanol (1 mg/mL). Then dilutions were performed to obtain concentrations (mg/mL) 0.033, 0.067, 0.133, 0.199, 0.266, 0.333, and 0.400, using each dissolution media in volume (seven levels). Each prepared solution was read in triplicate on a UV-visible spectrophotometer (Perkin Elmer Lambda 365®) at a wavelength of 475 nm. The experiment was carried out in triplicate. The linear regression equation for pH 1.2 was $Y = 1.5615X + 0.0115$, with a value of $r^2 = 0.9985$. The linear regression equation for pH 4.5 was $Y = 3.2513 - 0.0079$, with a value of $r^2 = 0.9989$. Similarly, the linear regression equation for pH 6.8 was $Y = 3.0093 - 0.0171$, with a value of $r^2 = 0.9993$. The working range for the three-dissolution media was 0.033 mg/mL and 0.44 mg/mL. The percentage of rifampicin present in each temporal sample of the dissolution media was determined from the concentrations obtained using the corresponding calibration curves.

Quantification of isoniazid

To quantify temporarily dissolved isoniazid, a spectrophotometric analytical methodology was designed and validated in the ultraviolet range for each dissolution media (pH 1.2, 4.5, and 6.8). The wavelength of maximum absorbance for the three media was 263 nm.

A calibration curve was prepared from a solution of the secondary standard of isoniazid in water (1 mg/mL). Then dilutions were performed to obtain concentrations (mg/mL) of 0.0222, 0.0444, 0.0667, 0.00889, 0.1111, and 0.1333, using each dissolution media in volume (six levels). Each prepared solution was read in triplicate on a UV-visible spectrophotometer (Perkin Elmer Lambda 365®) at a wavelength of 263 nm. The experiment was carried out in triplicate. The linear regression equation for pH 1.2 was $Y = 6.1009 - 0.0058$, with a value of $r^2 = 0.9999$. The linear regression equation for pH 4.5 was $Y = 5.2653 + 0.0025$, with a value of $r^2 = 0.9999$. For pH 6.8, the linear regression equation was $Y = 5.1650 + 0.002$, with a value of $r^2 = 0.9999$. The working range for the three media was 0.022 mg/mL and 0.1333 mg/mL.

Quantification of pyrazinamide

To quantify temporarily dissolved isoniazid, a spectrophotometric analytical methodology was designed and validated in the ultraviolet range for each dissolution media (pH 1.2, 4.5, and 6.8). The wavelength of maximum absorbance for the three media was 268 nm (Saldaña-Bobadilla et al., 2024).

A calibration curve was prepared from a solution of the secondary standard of pyrazinamide in water (1 mg/mL). Then, dilutions were made to obtain concentrations (mg/mL) of 0.056, 0.111, 0.222, 0.333, 0.444, 0.556, and 0.667, using each dissolution media in volume (seven levels). Each prepared solution was read in triplicate on a UV-visible spectrophotometer (Perkin Elmer Lambda 365®) at a wavelength of 268 nm. The experiment was carried out in triplicate. The linear regression equation for pH 1.2 was Y = 1.1504 - 0.0033, with a value of r^2 = 0.9981. The linear regression equation for pH 4.5 was Y = 1.1014 + 0.0076, with a value of r^2 = 0.9982. For pH 6.8, the linear regression equation was Y = 1.089 + 0.005, with a value of r^2 = 0.9999. The working range for the three media was 0.056 mg/mL and 0.667 mg/mL.

Dissolution profiles

Rifampicin capsules 300 mg

The dissolution profiles were carried out with twelve capsules of each multisource rifampicin formulation (RA and RB), using a Sotax AT7 Smart dissolution kit, USA, in three pH media (1.2, 4.5 and 6.8), according to the following conditions: Apparatus 1 at a rotation speed of 100 rpm, dissolution media 900 mL, media temperature 37 ± 0.5°C. For the test at pH 1.2, a 5 mL sample was taken from each glass at times of 5, 10, 15, 20, and 30 minutes without replacing the volume taken. For tests at pH 4.5 and 6.8, a 5 mL sample was taken from each glass at times of 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes without replacing the volume taken. All absorbance readings were done at 475 nm in a UV-visible spectrophotometer (Perkin Elmer Lambda 365®).

Isoniazid tablets 100 mg

The dissolution profiles were carried out with twelve tablets of each multisource isoniazid formulation (IA and IB), using a Sotax AT7 Smart dissolution kit, USA, in three pH media (1.2, 4.5, and 6.8), according to the following conditions: Apparatus 2 at a rotation speed of 75 rpm, dissolution media of 900 mL, media temperature of 37 ± 0.5°C. For tests at pH 1.2, 4.5, and 6.8, A 10 mL sample was taken from each glass at times of 5, 10, 15, 20, 30, and 45 minutes without replacing the volume taken. All absorbance readings were made at 263 nm in a UV-visible spectrophotometer (Perkin Elmer Lambda 365®).

Pyrazinamide tablets 500 mg

The dissolution profiles were carried out with twelve tablets of each multisource formulation of pyrazinamide (PA, PB, and IC), using a Sotax AT7 Smart dissolution kit, USA, in three pH media (1.2, 4.5, and 6.8), according to the following conditions: Apparatus 2 at a rotation speed of 75 rpm, dissolution media of 900 mL, media temperature of 37 ± 0.5°C. For tests at pH 1.2, 4.5, and 6.8, a 5 mL sample was taken from each glass at times of 5, 10, 15, 20, 30, 45, and 60 minutes without replacing the volume taken. All absorbance readings were made at 268 nm in a UV-visible spectrophotometer (Perkin Elmer Lambda 365®).

Determination of the similarity of dissolution profiles

All temporal concentrations of the dissolved drugs were transformed into percentages dissolved at each time.

To infer similarity, the similarity factor (*f2*) (MINSA, 2018a; Moore and Flanner, 1996) was used. This is a logarithmic transformation of the reciprocal of the square root of the sum of the squares of the errors and is a measure of the similarity of the dissolution percentages between two dissolution profiles. Thus, the WHO (WHO, 2017) and the FDA (2017) establish that two dissolution profiles will be considered similar if the value of *f2* is between 50 and 100. the similarity factor was calculated using the equation [1].

$$f_2 = 50.log\left(\frac{1}{\sqrt{1+\frac{\sum_{t=1}^{n}(R-T)^2}{n}}}.100\right) \quad [1]$$

Where: n: It is the number of sampling points. R: Dissolution value at each sampling point for the reference formulation. T: It is the dissolution value at each sampling point for the test formulation.

Statistical analysis

The percentages and temporal amounts dissolved were evaluated by their arithmetic mean, standard deviations, and their variation by the percentage coefficient of variation (CV), together with the number of observations (n) (Bolton and Bon, 2009). Calculations were carried out using a spreadsheet from the Microsoft Excel program version 2409, year 2022.

RESULTS AND DISCUSSION

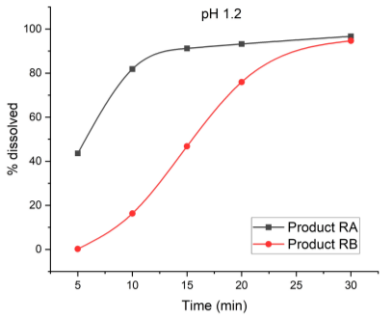
Protocols for the treatment of tuberculosis require drugs of biopharmaceutical quality that ensure that

therapeutic concentrations are within the expected therapeutic range, in a sustained manner. Any change in the characteristics of these medications could lead to therapy failure and increased bacterial resistance. In Peru, anti-tuberculosis drugs are acquired only by the state through tenders, with the result that they are not always purchased from the same pharmaceutical laboratory, a situation that affects a change in their quality, so it is necessary to ensure that they are biopharmaceutically similar. An alternative to evaluate this similarity is biowaiver studies (Strauch et al., 2011).

Table 1 shows the dissolution profiles of rifampicin in 300 mg capsules of the multisource products RA and RB, in dissolution media at pH 1.2, 4.5 and 6.8. The differences between their profiles are evident, highlighting that at pH 1.2, the dissolved percentages were higher compared to the other media for both formulations. Such is the case of pH 4.5, where the percentage of dissolution values was very low, even less than 50%. Furthermore, it is evident that the RA product had a faster dissolution at pH 1.2. However, it was slower at pH 4.5 and 6.8 compared to RB. The excipient present in the RA formulation that would impact rifampicin dissolution is sodium lauryl sulfate (FDA, 2017; Metry and Polli, 2022), a surfactant that is effective at acidic or basic pH. The RB product does not contain sodium lauryl sulfate. However, it contains sodium starch glycolate, a powerful disintegrant (Rowe et al., 2009). The dissolution percentages of rifampicin capsules (RA and RB) are also presented, observing that at 5 minutes at pH 1.2, RA dissolved 43.59%; however, RB at 0.18%, and 30 minutes, RA dissolved 96.71% and RB at 94.65. In pH 4.5 media, at 5 minutes, RA dissolved 8.8 % and RB 0.75%, and at 120 minutes, RA 30.71% and RB 44.30%. In the same sense, at 5 minutes at pH 6.8, RA dissolved 16.66% and RB 0.00%, and at 120 minutes, RA 53.11% and RB 81.69%. This same table indicates the similarity factor f2, 19.29 for pH 1.2, 50.60 for pH 4.5, and 32.16 for 6.8.

Table 1. Averages of dissolution percentages and similarity factor (f2) of 300 mg capsules of rifampicin from multiple sources RA and RB (n = 12).

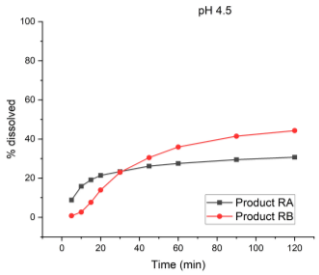
Dissolution media pH 1.2



Minutes	RA		RB		f_2
	% dissolved	C.V.	% dissolved	C.V.	
5	43.59	13.15	0.18	161.97	
10	81.84	4.78	16.33	13.96	RB/RA
15	91.21	4.94	46.79	18.24	19.29
20	93.21	5.64	75.93	8.82	*nf_2: 5
30	96.71	3.64	94.65	3.14	

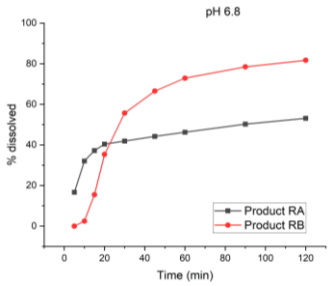
Table 1. Averages of dissolution percentages and similarity factor (f2) of 300 mg capsules of rifampicin from multiple sources RA and RB (n = 12) (continued...)

Dissolution media pH 4.5



Minutes	RA		RB		f_2
	% dissolved	C.V.	% dissolved	C.V.	
5	8.82	17.83	0.75	6.23	
10	15.82	10.04	2.65	16.35	
15	19.13	8.16	7.66	15.50	
20	21.40	8.60	13.95	10.58	RB/RA
30	23.34	3.93	23.00	8.77	50.60
45	26.17	5.26	30.50	7.88	*nf_2: 9
60	27.55	5.12	35.91	6.32	
90	29.46	4.59	41.45	5.41	
120	30.71	4.37	44.30	5.81	

Dissolution media pH 6.8



Minutes	RA		RB		f_2
	% dissolved	C.V.	% dissolved	C.V.	
5	16.66	22.00	0.00	-	
10	32.07	13.11	2.49	44.69	
15	37.23	7.02	15.46	21.31	
20	40.41	7.16	35.33	14.58	RB/RA
30	41.89	5.75	55.67	9.23	32.16
45	44.19	6.01	66.51	4.90	*nf_2: 9
60	46.24	5.27	72.89	4.06	
90	50.22	5.23	78.45	3.80	
120	53.11	4.22	81.69	4.15	

% dissolved: dissolved percentage of drug; C.V.: coefficient of percentage variation; RA: multisource rifampicin RA; RB: multisource rifampicin RB, *nf_2 times based to calculated f2.

According to international guides to establish similarity, *f2* must be between 50 and 100 in the three-dissolution media, or after 15 minutes, both products (comparator and the multisource dosage forms) have dissolved by 85% or more (ICH, 2019; WHO, 2017), showing that, for the two conditions, in this case, RA and RB are not similar, with the consequences that, by exchanging them, the desired therapeutic equivalence would not be achieved.

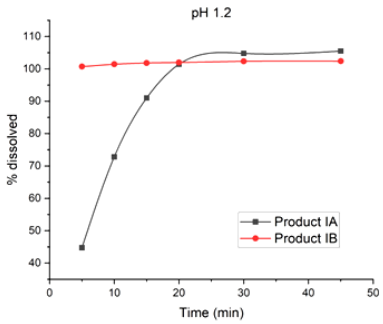
These differences between both products could be better explained by the type of rifampicin crystal and the particle size rather than by its manufacturing process or the components of its formulation. It has been reported that rifampicin can be presented in two crystalline forms (I and II) and one amorphous, with form II being the one recommended for pharmaceutical use. This limited solubility has led to the classification of rifampicin, according to the biopharmaceutical classification system (BCS), as class II, with low solubility and high permeability (Becker et al., 2009).

Given this poor solubility, Anjani et al. (2022) investigated the impact of the rifampicin-cyclodextrin complex on its solubility, concluding that this complex would be an alternative to improve the solubility of rifampicin and increase its bioavailability.

For the case of isoniazid in 100 mg tablets, Table 2 expresses a notable difference between the dissolution profiles of the IA and the IB in the three dissolution media at pH 1.2, 4.5, and 6.8. The IB product reached its maximum dissolution at 5 minutes. However, the IA did so at 20 minutes. However, the similarity between the profiles of the IA and the IB in the three dissolution media was due to the fact that isoniazid is highly soluble regardless of pH, so, depending on the BCS, it is class I or III, both with high solubility (Becker et al., 2007).

Table 2. Average dissolution percentages and similarity factor (f2) of 100 mg multisource IA and IB isoniazid multisource tablets (n = 12).

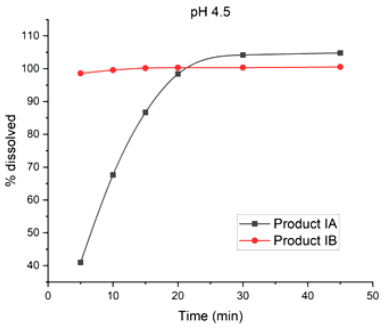
Dissolution media pH 1.2



Minutes	IA		IB		f_2
	% dissolved	C.V.	% dissolved	C.V.	
5	44.75	3.54	100.68	3.14	
10	72.79	3.00	101.41	3.03	
15	91.01	2.69	101.80	3.05	IB/IA
20	101.42	2.97	101.97	3.16	21.68
30	104.76	2.50	102.33	3.18	*nf_2: 3
45	105.48	2.48	102.37	3.12	

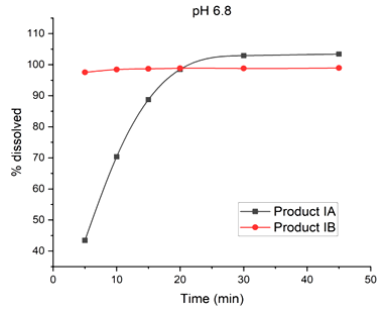
Table 2. Average dissolution percentages and similarity factor (f2) of 100 mg multisource IA and IB isoniazid multisource tablets (n = 12) (continued...)

Dissolution media pH 4.5



Minutes	IA		IB		f_2
	% dissolved	C.V.	% dissolved	C.V.	
5	40.95	5.64	98.56	3.56	
10	67.63	4.34	99.56	3.69	
15	86.68	4.73	100.16	3.66	IB/IA
20	98.38	3.80	100.31	3.61	20.54
30	104.17	2.94	100.32	3.52	*nf_2: 3
45	104.76	2.92	100.50	3.46	

Dissolution media pH 6.8



Minutes	IA		IB		f_2
	% dissolved	C.V.	% dissolved	C.V.	
5	43.45	4.02	97.53	2.29	
10	70.36	4.31	98.42	2.06	
15	88.74	3.20	98.68	2.16	IB/IA
20	98.49	2.86	98.83	2.11	22.39
30	102.90	2.50	98.79	2.10	*nf_2: 3
45	103.41	2.54	98.92	2.07	

% dissolved: dissolved percentage of drug; C.V.: coefficient of percentage variation; IA: multisource isoniazid IA; IB: multisource isoniazid IB, *nf_2 times based to calculated f2.

Although two new metastable polymorphs of isoniazid are reported, considered monomorphic for 60 years, these would have no effect on its solubility (Zhang et al., 2020).

The WHO considers biowaiver studies acceptable for products containing isoniazid since it has been shown to have very rapid dissolution (Becker et al., 2007; WHO, 2022). However, taking into account its classification in the Biopharmaceutical Classification

System (Class I or III), the formulation must be carefully evaluated. In this sense, all excipients used in both formulations must not compromise the bioavailability of the drug (Becker et al., 2008; ICH, 2019; Metry and Polli, 2022).

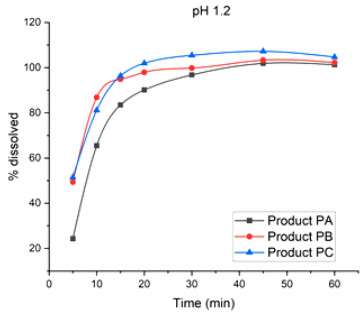
Regarding the dissolved percentages of the 100 mg isoniazid IA and IB tablets, they are described in Table 2, showing that at 15 minutes at pH 1.2, the IA dissolved 91.01%, while the IB dissolved 101.80%. In pH 4.5 media, the IA dissolved 86.68%, and the IB 100.16%. Similarly, at pH 6.8, the IA dissolved at 88.74% and the IB at 98.68%. In addition, the values of the similarity factors f2 obtained are presented: 21.68 for pH 1.2, 20.54 for pH 4.5, and 22.39 for 6.8. Although the f2 indeed was less than 50, and it could be inferred that they are not equivalent, this parameter should not be considered since the prevailing criterion is that both products dissolve more than 85% in the media of three dissolutions after 15 minutes (ICH, 2019; WHO, 2017), concluding that they are similar and therefore therapeutic equivalents, and can be exchanged.

Gwaza et al. (2014) investigated the differences in bioavailability between anti-tuberculosis generics prequalified by the World Health Organization through indirect comparisons to ensure their interchangeability. Among them, isoniazid, which was reported to be bioequivalent and, therefore, could be interchanged without safety and efficacy problems. Although it is true that these studies were carried out *in vivo*, they would be correlated with the *in vitro* studies carried out in this investigation.

Considering BCS, pyrazinamide is class III, with linear absorption in a wide dose range, so its risk of non-bioequivalence inferred through biowaiver studies is low (Becker et al., 2008). However, caution is important as pyrazinamide has a narrow therapeutic index. Table 3 presents the dissolution profiles over time for 500 mg pyrazinamide tablets of the PA, PB, and PC products, tested in media with pH 1.2, 4.5, and 6.8. It is observed that the PA product shows the lowest dissolution profile compared to the PB and PC products, which show similar profiles in the three dissolution media. It should be noted that the PA product uses croscarmellose sodium as a disintegrant, unlike the PB and PC products, which contain sodium starch glycolate, a more potent disintegrant, which could explain the difference in dissolution rate between PA and the other two products (Rowe et al., 2009).

Table 3. Averages of dissolution percentages and similarity factor (f2) of 500 mg tablets of PA, PB, and PC pyrazinamide from multiple sources (n = 12).

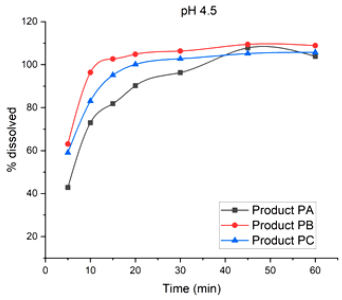
Dissolution media pH 1.2



Minutes	PA		PB		PC		f_2
	% dissolved	C.V.	% dissolved	C.V	% dissolved	C.V.	
5	24.28	21.80	49.35	27.85	51.47	11.96	PB/PA
10	65.81	10.26	86.81	8.07	81.17	7.43	37.39 *nf_2: 4
15	83.50	3.94	94.87	4.90	96.34	7.28	PC/PA
20	90.15	3.78	97.94	4.36	101.93	5.18	37.26 *nf_2: 4
30	96.77	1.67	99.81	3.97	105.44	6.29	PC/PB
45	101.90	2.70	103.31	3.17	107.20	6.61	71.46 *nf_2: 3
60	101.26	3.07	102.23	3.21	104.67	6.71	

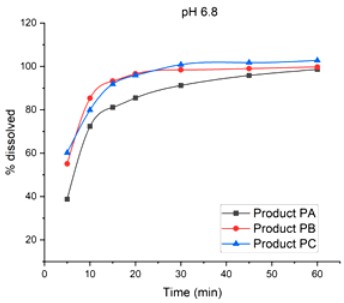
Table 3. Averages of dissolution percentages and similarity factor (f2) of 500 mg tablets of PA, PB, and PC pyrazinamide from multiple sources (n = 12) (continued...)

Dissolution media pH 4.5



Minutes	PA		PB		PC		f_2
	% dissolved	C.V.	% dissolved	C.V	% dissolved	C.V.	
5	42.83	20.98	63.05	27.28	59.03	11.19	PB/PA
10	72.96	11.41	96.36	6.74	83.07	6.76	34.89 *nf_2: 4
15	81.84	9.94	102.65	4.81	95.13	5.54	PC/PA
20	90.21	7.25	104.85	3.95	100.15	5.21	4.82 *nf_2: 4
30	96.30	4.92	106.32	3.80	102.77	4.66	PC/PB
45	107.84	6.85	109.44	4.00	105.15	5.88	51.87 *nf_2: 3
60	103.80	3.93	108.87	4.49	105.71	4.46	

Dissolution media pH 6.8



Minutes	PA		PB		PC		f_2
	% dissolved	C.V.	% dissolved	C.V	% dissolved	C.V.	
5	38.77	21.54	55.09	13.77	60.18	11.58	PB/PA
10	72.34	9.13	85.33	6.21	79.89	5.16	45.41 *nf_2: 5
15	81.11	8.29	93.27	3.01	91.87	4.38	PC/PA
20	85.49	6.08	96.59	2.17	95.87	4.55	44.41 *nf_2: 5
30	91.21	4.09	98.40	2.11	100.83	3.91	PC/PB
45	95.81	3.98	98.99	1.41	101.73	2.94	67.39 *nf_2: 3
60	98.63	3.46	99.74	2.57	102.77	5.42	

%: dissolved percentage of drug; C.V.: coefficient of percentage variation; PA: multisource pyrazinamide PA; PB: multisource pyrazinamide PB and, PC: : multi-source pyrazinamide PC, *nf_2 times based to calculated f2.

International guidelines on biowaiver studies are conservative for class III drugs, such as pyrazinamide, focusing their attention on the influence of excipients on absorption, recommending that the products to be compared should have the same excipients in similar quantities (Butler and Augustijns, 2022; ICH, 2019; WHO, 2017).

The dissolution percentages of the pyrazinamide tablets at different pH values are detailed in the same table. At pH 1.2, the PA tablet was observed to have dissolved 94.87% and 96.34%, respectively. In a medium with pH 4.5, the dissolution percentages were 81.84% for PA, 102.65% for PB, and 95.13% for PC, all after 15 minutes. At pH 6.8, PA was dissolved at 81.11%, PB at 93.27%, and PC at 91.87% in the same time interval. Furthermore, the f2 values are presented, which are lower than 50 in the PB/PA and PC/PA comparisons (4.82–45.41), while they are higher than 50 in the PC/PB comparison (51.87–71.46). These results suggest that PB and PC are equivalent in terms of dissolution, but PA differs significantly from both. In summary, PA does not reach 85% dissolution at 15 minutes compared to PB and PC, which exceed 85% in all three dissolution media tested. Therefore, it can be inferred that PB and PC tablets are interchangeable with each other, but PA is not (ICH, 2019; WHO, 2017).

Wang et al. investigated the bioequivalence between two formulations of 500 mg tablets of pyrazinamide, manufactured in China, with 24 subjects, in a fasting and postprandial state, concluding that the two products, in both states, were bioequivalent and therefore interchangeable (Wang et al., 2022).

Limitations of the study

Biowaiver studies for rifampicin require a greater number of units to be tested due to its high variability in its dissolved percentages, as it is a class III drug, poorly soluble; therefore, *in vivo* bioequivalence studies should be promoted.

CONCLUSION

Multisource rifampicin products did not prove to be *in vitro* biopharmaceutical equivalence to each other, while isoniazid products were. Furthermore, in the case of pyrazinamide, two products were found to be equivalent, while one was not.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Rodríguez-Hidalgo LA	Alva-Plasencia PM	Concepción-Urteaga LA	Hilario-Vargas J	Caballero-Aquiño OE	Saldaña-Bobadilla V	Vega-Fernandez AG
Concepts or ideas	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	
Literature search	x	x	x		x	x	x
Experimental studies	x	x				x	
Data acquisition	x					x	
Data analysis	x						
Statistical analysis	x	x	x			x	
Manuscript preparation	x	x	x	x	x	x	x
Manuscript editing	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x

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