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In Vitro Bioequivalence of Acetylsalicylic Acid and Implications in Public Health

María R. Bendezú[1], Jorge A. García[1], Haydee Chávez[1], Ana María Muñoz[2], John Jáuregui[3], Miguel Mucha[3], Ricardo Losno[4], María Saravia[4], and Angel T. Alvarado[4*]

[1]Faculty of Pharmacy and Biochemistry, San Luis Gonzaga National University of Ica, Ica, Peru.
[2]Institute of Food Science and Nutrition, ICAN, San Ignacio de Loyola University, Lima, Peru.
[3]Environmental Engineering, San Ignacio de Loyola University, Lima, Peru.
[4]International Network for Research in Pharmacology and Precision Medicine (REDIFMEP), School of Human Medicine, San Ignacio de Loyola University, Lima, Peru.

E-mail: eaa.alvarado@hotmail.com

ABSTRACT

Acetylsalicylic acid is one of the most prescribed medications in the world. It is used for prevention of acute myocardial infarction, colorectal cancer, antipyretic, and as an analgesic. This study aimed to investigate the in vitro bioequivalence of three formulations of acetylsalicylic acid, simulating physiological conditions of the dissolution medium, and analyzing its possible implication for public health. A spectrophotometric method for the quantification of acetylsalicylic acid at 265 nm was used. The dissolution test was performed using a USP apparatus 2 (paddle) with 900 mL of medium at 37 ± 0.5 °C and 75 rpm. At pH 4.5 and 6.8, the three formulations did not meet the criteria of very fast or fast dissolution (85% in ≤ 15 or 30 min, respectively). Generic A has a similarity factor (f_2) of 50 at pH 4.5 and 80.7 at pH 6.8; generic C f_2 values were 31.2 at pH 4.5 and 72.4 at pH 6.8. Generic B did not meet the acceptance range of the similarity factor (50–100) at pH 4.5 and 6.8. For all products tested, the dissolution efficiency was greater than 79%, and the mean dissolution time was 5.5–15.9 min. Based on the in vitro dissolution results, Generic A is bioequivalent with the innovator, whereas generics B and C are not. However, the dissolution profiles of generics A and C are similar to the innovator at pH 6.8, which is the appropriate dissolution medium for this drug.

KEYWORDS: Acetylsalicylic acid, generics, bioequivalent drug, dissolution profile, public health, dissolution

INTRODUCTION

The bioequivalence of generic drugs can be assessed through relative bioavailability studies and in vitro studies. Relative bioavailability studies apply to drugs with a high health risk and Biopharmaceutical Classification System (BCS) class 2 (low solubility and high permeability) and class 4 (low solubility and low permeability) drugs (*1-4*). In vitro bioequivalence studies apply to class 1 (high solubility and high membrane permeability) and class 3 (high solubility and low membrane permeability) drugs, the same test that is carried out in dissolution media simulating physiological pH (*2-4*).

Acetylsalicylic acid (ASA, 2-acetoxybenzoic acid, $C_9H_8O_4$), is a BCS class 1, non-steroidal anti-inflammatory drug (NSAID). Due to the presence of a carboxylic group, ASA has a pKa of 3.5, so its solubility depends on the pH, and it is partially absorbed into the gastric mucosa and

*Corresponding author

mainly in the duodenal mucosa (*5*, *6*). In addition, 75% of the absorbed drug undergoes a presystemic effect or a first-pass effect on the intestinal wall (intestinal esterases) and in the liver (*7*). The plasma level is dependent on the dose, ranging from 50–100 mg/L (1.3–2.6 g/day) for an analgesic and antipyretic effect or 125–300 mg/L (4–6 g/day) for an analgesic-anti-inflammatory effect, with a maximum time (t_{max}) of 1 hour (*7*). The salicylate form circulates mainly bound to plasmatic albumin in 80–90%, and its volume of distribution is 0.1–0.2 L/kg, which indicates moderate diffusion to tissues and liquids, such as breast milk and cerebrospinal fluid (*8*). Due to the presystemic deacetylation reaction, ASA dissociates into an acetyl cation (deacetylated metabolite responsible for the mechanism of action), and a salicylic anion reacts with the proton (H^+) of serine 529 of platelet COX-1 to form salicylic acid; this acid at the liver level is metabolized by phase I and II (*9*). By phase I, oxidation gentisic acid is formed (< 1%) (*7*). By phase II of conjugation with glycine, it forms salicyluric acid (75%, rapid and saturable reaction at doses greater than 650 mg of ASA, elimination of zero-order, and could generate toxicity); with UDP-glucuronic acid it forms salicylphenolic glucuronide ether (10%) and salicylacyl glucuronide ester (5%) (*7*). With a dose greater than 1 g, the ether conjugate saturates and the kinetics will be zero-order, increasing the half-life of the salicylate in plasma (*7*). Its elimination half-life ($t_{1/2}$) is 2–3 hours at a single analgesic dose with first-order kinetics; at repeated doses, it is 5–30 hours (*8*). Ten percent of salicylate is eliminated by the urine without being metabolized, and 75% as salicyluric acid (*7*).

The pharmacological effects of ASA depend on the dose and the mechanism of action on cyclooxygenase (COX) enzymes (*10, 11*). At doses of 40–80 mg/day, ASA in the blood is deacetylated, giving rise to the acetyl cation that binds to the residue of arginine-120 and acetylates the hydroxyl group (-OH) of serine 529 of platelet COX-1, forming a covalent and irreversible bond, so platelets cannot synthesize new COX-1 during their half-life of 7–10 days (*9*). This antiplatelet effect is used in the prevention of cardiovascular events, and it can prevent colorectal cancer, but it predisposes to gastrolesivity and bleeding, due to decreased PGE_2 and PGI_2; at higher doses (325–650 mg/4–6 hours) ASA inhibits COX-2, relieving pain, inflammatory processes, and fever, but blocks the vasodilator and antiplatelet effects on the vascular wall (*6, 11–17*).

In developing Latin American countries, it is essential to carry out these studies for three reasons. First, in vitro bioequivalence studies must be carried out on BCS class 1 test and reference drugs that dissolve 85% of the active pharmaceutical ingredient (API) within 15 or 30 minutes (*2, 4, 18*). Second, to help ensure the quality of medicines used by the Ministry of Health, which ensures adequate dissolution, absorption, and therapeutic plasma levels (*19, 20*). Thirdly, identification of medicines from dubious or falsified sources, i.e., medicines without API or with insufficient amounts of API has been reported as a public health concern at the international level (*3, 21*). Having bioequivalent and interchangeable generic drugs in health facilities allows patients to have access to good quality and affordable drugs, and at the same time, supports to the country's public health policies.

The objective of this research was to investigate the in vitro bioequivalence of three formulations of ASA, simulating physiological conditions of dissolution at three pH levels, and analyzing the possible implications for public health.

MATERIAL AND METHODS

The study was an analytical, experimental, cross-sectional, and double-blind design, conducted to comply with applicable compendial specifications (*3, 4, 18, 20, 22*).

Chemical Reagents

Reagents of analytical grade and American Chemical Society quality were used. The following were purchased from Mercantil Laboratory SAC (Lima, Peru): hydrochloric acid (HCl) 36%, anhydrous sodium acetate (CH_3-COONa), sodium hydroxide (NaOH), monobasic potassium phosphate (KH_2PO_4), and United States Pharmacopeia (USP) acetylsalicylic acid standard. These reagents were kept under analytical laboratory conditions (25 °C, 60% relative humidity).

Study Design and Samples

Two hundred ASA immediate-release tablets (100 mg), including three generic products and one innovator brand (Aspirin), were purchased at a local pharmacy in Ica, Peru that is common throughout the country. All samples were registered with the General Directorate of Medicines, Supplies, and Drugs (DIGEMID). The samples were stored on closed shelves, protected from light, under laboratory conditions (25 °C, 60% relative humidity).

Each formulation was randomly identified with letters: generic A (lot 2013361, exp 07/2023, LABPORT), generic B (lot 837101602, exp 06/2023, LABAMER), generic C (lot 1090354, exp 06/2023, LABFARM), and the innovator as R (lot NV-3456, exp 05/2023, Bayer).

Method Validation and Calibration

Specificity (to detect interference of the excipients with the active pharmaceutical ingredient), linearity (over the range of 1.60–7.75 µg/mL), and precision of the dissolution method were evaluated by spectrophotometry (Unico Model UV 2100 Series, USA) at 239 nm using six 50-mg propylthiouracil tablets.

USP Prednisone RS 10-mg tablets (lot R080J1) were used to calibrate the dissolution apparatus (Electrolab ETC-11Lx, model 1104197, series 1201044, India). The experimental conditions were: 500 mL of purified water as dissolution medium, 37 °C ± 0.5 °C, 75 rpm, for 30 min. The isothermal medium was qualified by setting the temperature selector at 37 °C, and uniformity of the heated water bath that heats the distilled water inside and outside the dissolution vessel was verified.

Weight Variation Determination

Twenty tablets of each ASA formulation were dedusted from the surface and individually weighed on an analytical balance (Boeco BBL31, Germany). A coefficient of variation less than 4% was established as the acceptance limit.

Hardness Test

Ten tablets of each ASA formulation were selected, then each tablet was placed in a durometer (BIOBASE THAT-3, China) to generate rupture. An acceptance limit of 6 ± 2 kgf was established.

Content Assay

For the content assay, the average weight of 20 tablets was determined, then crushed into a fine powder; 150 mg of powder was weighed, added to a 200-mL volumetric flask, then 50 mL of 0.1 N NaOH and 100 mL of distilled water were added. The mixture was subjected to ultrasound (Ultrasound, Lab Companion, UC-10, JT-11AB-078-YP series, Korea) for 15 minutes. The solution was left at laboratory temperature for 15 minutes, then distilled water qsp 200 mL (Solution A) was added. With a volumetric pipette, 10 mL of solution A was measured, filtered at the time of transfer to a 100-mL volumetric flask, to which distilled water qsp 100 mL was added (Solution B). Then 10 mL of solution B was measured and transferred to a 100-mL volumetric flask, to which 10 mL of 0.1 N NaOH was added, mixed, and made up to volume with distilled water qsp to obtain a final concentration of 7.5 µg/mL. Both the blank (distilled water) and the sample were read at a wavelength of 265 nm. The analysis was performed in triplicate (*23, 24*).

Dissolution Profile

Twelve tablets were used for each generic formulation (A, B, and C) and reference (R) of ASA to evaluate the dissolution profile. The experimental conditions were: USP apparatus 2 (paddle), 900 mL of dissolution medium at pH 1.2, 4.5, and 6.8, 37 ± 0.5 °C, 75 rpm, and 8 pre-established sampling times (5, 10, 15, 20, 25, 30, 45, and 60 min), 5-mL sample volume, and no medium replacement.

The absorbances of the blank (dissolution medium according to pH) and the samples were determined by spectrophotometry at 265 nm. To determine the concentration and percentage of dissolution, a calibration curve (R^2 = 0.99) was used (*22*).

Statistical Analysis

As a statistical indicator of in vitro therapeutic equivalence, dissolution efficiency (DE%), mean dissolution time (MDT), and similarity factor (f_2) were used. If more than 85% of the API was dissolved in less than 15 minutes, the dissolution profiles would be accepted as similar without further mathematical evaluation, i.e., without using the f_2. Microsoft Excel was used to calculate the results.

RESULTS

The quality control parameters that influence the biopharmaceutical phase of the tablets, such as variation in weight, hardness, and content, were within the USP acceptance criteria (*23, 24*). These results are presented in Table 1.

Table 2 and Figure 1 show that the ASA formulations at pH 1.2 dissolved more than 85% in less than 15 minutes, so it was not necessary to apply the f_2 analysis. At pH 4.5, generic A released 85.17% of API at 60 minutes, compared to the innovator which released more than 85% at 45 minutes. At pH 6.8, drug release was less than 85% at up to 60 minutes of the experiment for the three generic products investigated.

Table 3 describes the parameters that characterize the API release curve for 100-mg ASA immediate-release tablets. The f_2 value for generic A is in the acceptance range of 50–100 at pH 4.5 and 6.8, whereas generic C is only equivalent at pH 6.8. Generic B does not exceed the minimum value of f_2. DE% was greater than 79.4% (acceptance criterion 63.2%) and

MDT was below 20 minutes at all three pH levels.

Table 1. Quality Control Parameters for 100-mg Acetylsalicylic Acid Immediate-Release Tablets (n = 20)

Product	Weight, mg (CV < 4%)			Hardness, kgf (6 ± 2 kgf)			Content, % (90–110%)	
	Mean	SD	CV%	Mean	SD	CV%	%	SD
Innovator	229.6	1.05	0.46	5.6	0.23	4.02	100.2	0.10
Generic A	120.1	1.90	1.58	4.2	0.16	3.81	100.5	0.55
Generic B	126.8	1.34	1.05	4.9	0.24	4.84	99.6	0.46
Generic C	127.6	1.76	1.38	4.8	0.19	3.99	100.1	0.14

SD: standard deviation; CV: coefficient of variation.

Table 2. Dissolution (% drug release) of 100-mg Acetylsalicylic Acid Immediate-Release Tablets

Time (min)	Innovator Brand			Generic A			Generic B			Generic C		
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
Dissolution medium: Hydrochloric acid, pH 1.2												
5	90.94	1.03	1.13	97.53	1.08	1.11	89.46	1.30	1.46	88.05	1.14	1.29
10	91.63	1.10	1.20	98.64	1.10	1.12	89.69	1.40	1.56	88.66	0.92	1.03
15	99.45	0.76	0.77	98.26	1.16	1.19	88.96	0.89	1.00	89.47	1.21	1.36
20	99.47	0.80	0.80	98.49	1.06	1.08	90.67	1.22	1.34	90.41	1.25	1.39
25	99.54	0.80	0.81	99.03	1.21	1.22	92.48	1.32	1.43	92.20	1.07	1.16
30	99.61	1.18	1.19	99.41	0.31	0.31	94.29	1.20	1.27	95.30	1.24	1.30
45	99.66	1.22	1.22	99.77	0.21	0.21	97.65	1.11	1.13	98.29	1.32	1.35
60	99.84	1.42	1.42	99.88	0.23	0.23	100.48	1.73	1.72	98.85	1.18	1.19
Dissolution medium: Acetate buffer, pH 4.5												
5	60.85	1.22	2.00	56.10	1.54	2.74	41.51	1.09	2.63	42.93	1.20	2.79
10	62.40	2.83	4.53	56.83	1.28	2.26	45.74	1.11	2.42	45.89	1.56	3.39
15	69.46	0.98	1.42	57.50	1.63	2.83	48.63	0.55	1.14	49.63	0.85	1.71
20	75.47	1.10	1.46	61.19	0.90	1.47	50.65	1.40	2.77	51.38	1.16	2.26
25	79.03	1.30	1.65	66.38	1.38	2.08	52.49	1.27	2.43	54.36	1.79	3.30
30	82.90	1.32	1.59	71.59	1.61	2.25	54.31	1.19	2.19	55.99	1.68	3.00
45	89.64	1.41	1.57	79.18	1.68	2.12	58.66	1.02	1.73	60.35	1.26	2.09
60	90.48	1.17	1.29	85.17	2.33	2.73	61.33	0.95	1.55	62.81	1.25	1.99
Dissolution medium: Phosphate buffer, pH 6.8												
5	46.83	0.85	1.82	46.16	1.09	2.35	41.54	1.09	2.61	41.35	1.22	2.94
10	49.37	0.37	0.74	48.32	0.82	1.69	45.72	1.05	2.30	47.70	0.85	1.79
15	57.26	0.90	1.57	56.29	1.42	2.52	48.40	0.89	1.84	52.18	1.08	2.08
20	63.62	1.93	3.03	63.55	1.23	1.94	50.95	1.30	2.56	61.39	1.19	1.94
25	69.32	1.24	1.78	75.21	1.62	2.16	52.81	1.40	2.65	66.68	1.44	2.16
30	76.98	1.74	2.26	78.46	1.30	1.65	54.14	0.68	1.26	72.68	1.38	1.90
45	82.80	1.28	1.55	80.67	0.93	1.16	57.53	0.92	1.60	80.00	1.25	1.57
60	82.85	1.75	2.12	81.25	1.08	1.33	58.50	1.16	1.98	81.43	1.49	1.83

SD: standard deviation; CV: coefficient of variation.

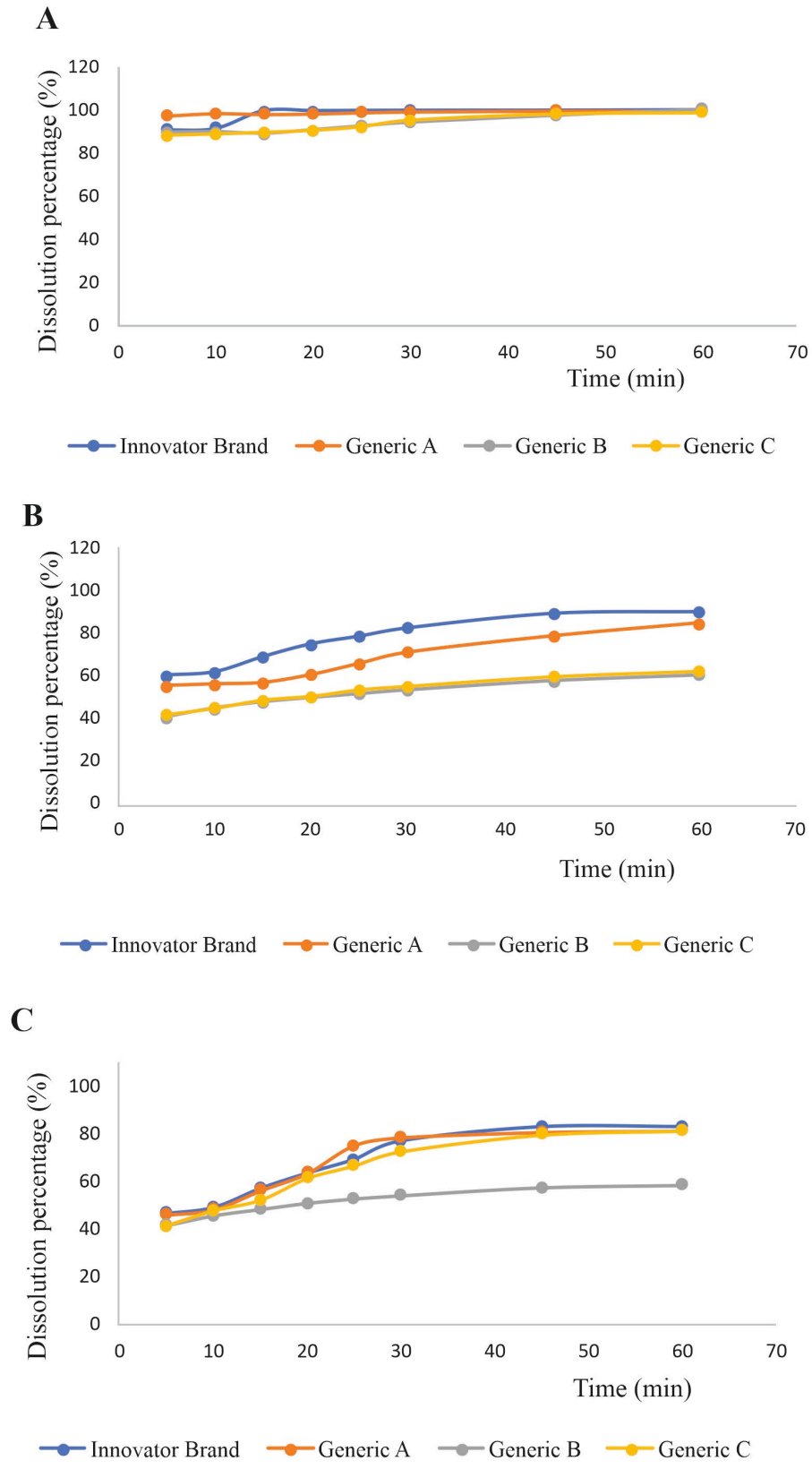

Figure 1. Dissolution profiles of 100-mg acetylsalicylic acid tablet formulations at pH 1.2 (A), 4.5 (B), and 6.8 (C).

Table 3. Characterization of Dissolution Profiles for 100-mg Acetylsalicylic Acid Immediate-Release Tablet Formulations at pH 1.2, 4.5, and 6.8

	f_2 (%)		AUC_o^t (min%)			DE (%)			MDT (min)		
Product	**4.5**	**6.8**	**1.2**	**4.5**	**6.8**	**1.2**	**4.5**	**6.8**	**1.2**	**4.5**	**6.8**
Generic A	50.0	80.7	5699.4	4032.4	4051.6	95.1	78.9	83.1	5.5	15.9	12.8
Generic B	30.0	38.5	5417.5	3078.0	3040.2	89.8	83.6	86.6	8.9	12.9	10.9
Generic C	31.2	72.4	5412.6	3157.1	3880.5	91.3	83.8	79.4	7.9	12.8	15.4
Innovator	-	-	5644.9	4588.3	4065.2	94.2	84.5	81.8	5.9	12.2	13.8

f_2: similarity factor; AUC_o^t: area under the curve by the trapezius method; DE: dissolution efficiency; MDT: mean dissolution time.

DISCUSSION

The three immediate-release generic products of ASA (100-mg tablets) from Peru used in this study met the official specifications for quality control tests. Risha et al. have also demonstrated that the formulations of ASA (100 mg) and other drugs met pharmacopoeial requirements for API content in Tanzania (*25*). Osorio et al. also reported that five formulations of ASA (100 mg) met the quality control parameters in Colombia (*22*).

After quality control testing, in vitro bioequivalence was evaluated at eight sampling points, simulating the fluid and the physiological peristaltic movement of the gastrointestinal tract. At pH 1.2, all ASA tablets dissolved by more than 85% in less than 15 minutes; however, at pH 4.5, all three generic ASA tablets did not meet the criteria of very fast-dissolving (release 85% of API in ≤ 15 min) or fast-dissolving drugs (release more than 85% in ≤ 30 min) (*2, 4, 18*). At pH 6.8, 80% API was released from all generic formulations A, C, and the innovator at 45 minutes (R 82.8%, A 80.67%, and C 80.0%); however, according to the *USP* acceptance criteria, ASA tablets must release 80% API at 30 minutes (*23, 24*).

These results are directly related to the Henderson-Hasselbach equation (pH = pKa + log [I/NI], due to pKa of ASA = 3.5); i.e., in a dissolution medium at pH 6.8, the drug will be ionized in its carboxylate form (I) with greater solubility and dissolution (*5, 26*). Thus, the in vitro dissolution medium that simulates gastric or intestinal fluid should be selected to predict an optimal dissolution profile (*5*). It is known that fasting gastric pH is acid (pH 1.2) and in the presence of food it can reach pH 4.9 (*27*). In the duodenum, the pH is 6.5 (fasting and in the presence of food), in the ileum the pH is 7.4 (*5*). Previous studies have shown that the dissolution medium influences API release, and Krieg et al. indicate that it is a determining factor of the biopharmaceutical phase (disintegration and dissolution) of oral solid pharmaceutical forms (*28*). Markopoulos et al. conclude that the dissolution rate of solid formulations depends on the pH, which affects the bioavailability of the drug (*29*). The study by Risha et al. indicates that three formulations of ASA did not meet the tolerance limits for dissolution, and Osorio et al. mentioned that one of five ASA formulations did not meet the dissolution test acceptance criteria (*22, 25*). However, Alemanni et al. have shown that the aspirin formulation dissolved more than 80% at 6 minutes at the three pHs (1.2, 4.5, and 6.8), which differs from our study (*30*).

In the present study, f_2 at pH 1.2 was not determined because the three formulations released more than 85% API in less than 15 minutes. However, it may be worth considering a study by Matiz et al. who indicated that if a generic formulation is “suprabioavailable,” then it can release a high amount of API that in vivo would indicate a high concentration of drug

in the biophase and be responsible for toxicity, so it would be incorrect to declare such drugs as bioequivalent (*31*). At pH 4.5, only generic drug A presented a dissolution profile like the innovator with a 10% difference (f_2 50); at pH 6.8, the dissolution profiles of generics A and C were similar to the innovator, exceeding values of 65, with a difference of less than 5% (f_2 A 80.7; C 72.4). The DE% of the ASA formulations at all three pH levels was greater than 79.4% (acceptance criterion 63.2%), indicating that the tablets will release an adequate amount of drug in the fluid that will be bioavailable in the intestinal mucosa for absorption (*4, 18, 20*). MDTs were between 5.5 (pH 1.2) and 15.9 minutes (pH 4.5), indicating that there is no correlation with mean gastric emptying (residence time), which under fasting conditions is between 15 and20 minutes. Based on the values of f_2, DE%, and MDT, generics A and C dissolve more efficiently at pH 6.8, so the use of an enteric-coated ASA tablet would be justified, to prevent the pharmaceutical form from dissolving at the gastric level (i.e., avoiding gastrolesivity), and when it reaches the small intestine, it dissolves and is absorbed to a greater extent (*5*).

Pharmaceutical technology (formulation, excipients, granulation, and type of compression), physicochemical properties of the drug (pKa and partition coefficient), and dissolution media in vitro and in vivo (gastrointestinal fluid of the patient and gastric emptying) all influence in absorption and bioavailability of ASA, generating an impact on the health of patients. If the drug does not reach the minimum effective concentration (C_{mE}), the risk of therapeutic failure increases, and if it exceeds the maximum effective concentration (C_{ME}) it predisposes to side reactions or toxicity of the drug. In both situations, the patient's illness is aggravated and the cost of treatment and hospitalization are increased, with negative repercussions for the country's public health system.

The limitations of this study are in the number of samples (n = 3 generic formulations) studied from the Peruvian pharmaceutical market. The friability, disintegration, and influence of excipients of each formulation on dissolution kinetics was not evaluated, so these are being considered for future research. However, we consider that the results are relevant, as they contribute to in vitro bioequivalence studies in the country and generate scientific evidence on the subject with an aim to guarantee the availability and access of interchangeable medicines for Peruvians.

CONCLUSION

Generic A is bioequivalent in vitro with the innovator, whereas generics B and C are not because the f_2 values differ in two dissolution media (at pH 4.5 and 6.8). However, the dissolution profiles of generics A and C are similar to the innovator at pH 6.8, which is the appropriate dissolution medium pH for ASA.

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CONFLICTS OF INTEREST

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

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