The AAPS Journal (© 2012)
DOI: 10.1208/s12248-012-9350-9

Research Article

Toward Global Standards for Comparator Pharmaceutical Products: Case Studies of Amoxicillin, Metronidazole, and Zidovudine in the Americas

Raimar Löbenberg,[1,4] Nadia B. Chacra,[2] Erika S. Stippler,[3] Vinod P. Shah,[3] Anthony J. DeStefano,[3] Walter W. Hauck,[3] and Roger L. Williams[3]

Received 30 November 2011; accepted 19 March 2012

Abstract. This study compared *in vitro* dissolution characteristics and other quality measures of different amoxicillin, metronidazole, and zidovudine products purchased in the Americas to a comparator pharmaceutical product (CPP). These three drugs are classified as Biopharmaceutics Classification System Class I drugs with the possibility that dissolution findings might be used to document bioequivalence. All investigated zidovudine products were found to be *in vitro* equivalent to the CPP. Only 3 of 12 tested amoxicillin products were found to be *in vitro* equivalent to the CPP. None of the tested metronidazole products were *in vitro* equivalent to the CPP. These findings suggest but do not confirm bioinequivalence where *in vitro* comparisons failed, given that an *in vivo* blood level study might have confirmed bioequivalence. At times, identifying a CPP in one of the selected markets proved difficult. The study demonstrates that products sold across national markets may not be bioequivalent. When coupled with the challenge of identifying a CPP in different countries, the results of this study suggest the value of an international CPP as well as increased use of BCS approaches as means of either documenting bioequivalence or signaling the need for further *in vivo* studies. Because of increased movement of medicines across national borders, practitioners and patients would benefit from these approaches.

KEY WORDS: bioequivalence; Biopharmaceutics Classification System; comparator pharmaceutical products; equivalence; standards.

INTRODUCTION

The World Health Organization (WHO) vision for essential medicines is "that people everywhere [should] have access to the essential medicines they need; that the medicines are safe, effective, and of assured quality; and that they are prescribed and used rationally" (1). Today, this remains a challenge in many developing countries partly because of counterfeit drugs (2) but also because of a lack of sufficient regulatory oversight to ensure drug quality (3,4). Multisource (generic) medicines help to make drug therapy more likely affordable, but they must be interchangeable, *i.e.*, therapeutically equivalent to an innovator product. The pharmaceutical and regulatory criteria for interchangeable multisource medicines in the US market are described in the *Orange ~~b~~ook* published by the Food and Drug Administration (FDA) (5) and in many other regulatory documents.

Generally, the first step in generic development in the USA is to create a product that is pharmaceutically equivalent to the Reference Listed Drug (RLD) specified in the *Orange ~~b~~ook*. FDA defines pharmaceutical equivalence as a drug product that:

1. contains the same active ingredient(s) and salt form,
2. uses the same dosage form and route of administration, and
3. has the same strength or concentration as the RLD.

The generic drug manufacturer then conducts relative bioavailability (bioequivalence) studies comparing the RLD and the proposed generic equivalent (5), typically using the listed innovator product. Clinical bioequivalence testing to establish therapeutic equivalence can be relatively expensive and time consuming. An alternative is dissolution testing to establish *in vitro* bioequivalence (6). This approach can be used for certain highly soluble drugs according to the Biopharmaceutics Drug Classification System (BCS) (7). Today, the science and validity of the BCS are well established, and many

Electronic supplementary material The online version of this article (doi:10.1208/s12248-012-9350-9) contains supplementary material~~, which~~ is available to authorized users.

[1] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada.
[2] Faculty of Pharmacy, University of São Paulo, São Paulo, São Brazil.
[3] US Pharmacopeial Convention, Rockville, Maryland, USA.
[4] To whom correspondence should be addressed. (e-mail: raimar@ualberta.ca)

Q2 Q1

biowaiver extensions have been proposed by the scientific community and some have been approved by regulatory bodies (8–11). Note: a dichotomy in nomenclature exists between WHO and US documents wherein bioequivalence in WHO terminology refers to a comparative blood level (pharmacokinetic studies). The USA allows a broader definition of the types of bioequivalence (BE) studies (also comparative clinical, pharmacodynamic, and *in vitro* studies). This paper uses the US terminology so that pharmaceutical equivalence and bioequivalence (with the several options available) equals therapeutic equivalence (12). WHO also uses the term comparator pharmaceutical product (CPP) instead of RLD.

Based on the BCS, WHO developed the *Proposal to waive in vivo bioequivalence requirements for WHO model list of essential medicines immediate-release, solid oral dosage forms* (6). This document outlines the criteria under which *in vitro* testing can replace *in vivo* bioequivalence testing. In brief, the proposal applies to drug products that contain BCS class 1 or 3 drugs and also to some class 2 drugs. A generic tested in three different media must have dissolution profiles that are similar to those of the comparator product. The aim of WHO's proposal is to enable regulatory agencies in developing countries to approve generics based on comparative *in vitro* studies instead of bioequivalence studies (8). The WHO proposal suggests using a well-established drug product, usually the innovator's product, as the CPP.

The current study identified the RLD or another suitable product listed in the *Orange book* as the CPP (5). FDA approved these products because they were shown to be safe and effective when used as directed. Furthermore, FDA requires that any postapproval manufacturing change must be shown by a manufacturer to maintain therapeutic equivalence to the prechange product (5).

The goal of the study reported here was to examine and document product performance of three widely used drug products marketed in different countries of the Americas. The study investigated the dissolution behavior of different amoxicillin, metronidazole, and zidovudine products purchased in those countries. The generic products were compared to the CPP and to each other to determine if they met *in vitro* bioequivalence criteria (8). The study hypothesis was that the different drug products would meet the criteria for *in vitro* equivalence. The dissolution studies presented in this report repeat the type of studies conducted by Blume *et al.* with the difference that BCS criteria were incorporated into the study design. With the understanding arising from the BCS, the studies in the present report can also signal bioequivalence, which is termed *in vitro* equivalence where applicable. *In vivo* studies were not performed in this study. Thus when *in vitro* studies did not signal bioequivalence, further clinical studies might have confirmed this conclusion.

METHODS

Chemicals

Amoxicillin Reference Standard (RS) (J0C043), Metronidazole RS (JOC316), and Zidovudine RS (HOF263) were received from US Pharmacopeia (USP, Rockville, MD). Acetonitrile, potassium phosphate, sodium acetate, and sodium hydroxide were purchased from Caledon (Georgetown, ON). Hydrochloric acid, potassium hydroxide, and phosphoric acid were received from Fisher Scientific (Bridgewater, NJ). All chemicals were USP or American Chemical Society grade.

Weight Variation

The weight of 18 capsules or tablets was recorded for each product tested. The weight variation was calculated as standard deviation(s) using Eq. 1:

$$s = \sqrt{\sum \frac{(X_i - \overline{X})^2}{n-1}} \quad (1)$$

where x_i are individual weights, $\overline{X}$ is the mean of all weights, and n is the number of samples measured. Weight variation was recorded to assess whether any analytical data would show abnormally high or low values linked to an overdosing or underdosing of the test units.

Content Uniformity

The chemical assay was performed for each CPP according to its USP monograph. If required by the CPP's USP monograph, Uniformity of Dosage Units <905> tests were performed. Analysts evaluated the content uniformity using an Excel spreadsheet published by USP (17).

Media Preparation

Simulated gastric fluid (SGF), acetate buffer pH 4.5 USP, and simulated intestinal fluid (SIF) were prepared according to instructions in USP Test Solutions. All media were prepared without enzymes. The density of each medium was determined at room temperature using a 1-L volumetric flask.

Media were deaerated in the following manner: 1 L dissolution medium was heated above 41 °C and filtered through a 0.45-μm filter (Fisher General Filtration MEC filter, 0.45 μm) into a media bottle that was immersed in a Branson Model 8200 ultrasonic bath (Brandson, Danbury, CT).

Table I lists all amoxicillin products tested, Table II all metronidazole products, and Table III all ziduvudine products. All products were tested at least 12 months before their stated expiry date.

Dissolution Test

A VK 7020 dissolution tester with six vessels and a VK 8000 autosampler station (Varian Inc., Carey, NC) was used. USP Apparatus 2 (paddle) at 75 rpm and 900 mL media were used for all tests. Preheated and degassed dissolution medium was weighed into each dissolution vessel individually. The filling process was performed with caution to avoid inclusion of air into the medium. The test was started after the temperature in all vessels was confirmed.

USP sinkers were used for the capsule products. Sample concentrations were determined via high-performance liquid chromatography (HPLC) analysis: 1.25 mL medium was withdrawn from each vessel at each time point and filtered (Full Flow Filters, Varian Inc.), and 1 mL was transferred into

Standards for Comparator Pharmaceutical Products

Table I. Amoxicillin Products Tested

Country	Company	Product	Batch	Expiry	Excipients
USA	Sandoz	Amoxicilin 500 mg	151645	09 Oct	Silicon dioxide, crospovidone, ethylcellulose aqueous dispersion, hypromellose, magnesium stearate, microcrystalline cellulose, sodium starch glycolate, talc, triethyl citrate, and titanium dioxide
Argentina	Roemmers	Amoxidal	633	10 Nov	Starch, crospovidone; sodium lauryl sulfate, magnesium stearate, microcrystalline cellulose, hypromellose, titanium dioxide, polyethylene glycol, and triacetine
	Klonal	Amox-G	A5802	10 Jan	Authorized excipients
	Bernabo	Amixen 500 mg	117183	09 Nov	Hypromellose, polyethylene glycol, crospovidone, magnesium stearate, microcrystalline cellulose, lactose, titanium dioxide, triacetine, and amaranthus
	Ahimsa	Amoxigrand	P213G911	10 Oct	Authorized excipients
	Sandoz	Telmox 500 mg	18	11 Jan	Magnesium stearate, microcrystalline cellulose, titanium dioxide, hydroxypropyl cellulose, povidone, and sodium carboxymethyl starch
Peru	Saval	Amoval	122387	12 Jul	Croscarmellose sodium, microcrystalline cellulose, magnesium stearate, titanium dioxide, polyethylene glycol, hypromellose, and eicosadioate
	Grünenthal (Trifarma)	Grunamox	9016	09 Sep	
	Farmindustria	Amoxicilina	921787	10 Sep	
Chile	Laboratórios Chile	Amobiotic	8016317	11 Jan	Povidone, sodium starch glycolate, microcrystalline cellulose, magnesium stearate, polymeric coating, talc, titanium dioxide, simeticone, macrogol, and hypromellose
	Laboratórios Chile	Amoxicilina LCh	7072912	10 Jul	
	Andromaco	Amoxicilina	1700408	09 Dec	
	Saval	Amoval 500 mg	33608	12 Nov	Croscarmellose sodium, microcrystalline cellulose, magnesium stearate, titanium dioxide, polyethylene glycol, hypromellose, and eicosadioate

a 2.5-mL vial for quantitation. The remaining fluid was discarded and media were not replaced in the vessels after sampling. Drug concentration was corrected by calculation for the withdrawn volume. The sampling time points were 10, 15, 20, 30, 45, and 60 min.

Analytical Quantitation

The amount of dissolved drug was determined using a HPLC method. The system comprised a system controller SCL-10A, two LC-10A pumps, an autosampler SIL-10ADvp,

Table II. Metronidazole Products Tested

Country	Company	Product	Batch	Expiry	Excipient
USA	Searle Pharmacia	Flagyl	C061228	38784	Cellulose, FD&C blue, hydroxypropyl cellulose, hypromellose, polyethylene glycol, stearic acid, and titanium dioxide
Argentina	Aventis	Flagyl	U6121	10 Oct	Water, ethanol, maize starch, calcium phosphate dihydrate, magnesium stearate, hypromellose, white wax, titanium dioxide, polyethylene glycol 20,000, povidone, and sorbitol anhydrate
	Lazar	Colpofilin	L0001	11 Feb	Lactose, microcrystalline cellulose, DOSS Na, povidone, croscarmellose sodium, talc, and magnesium stearate
	Baliarda	Ginkan	403	10 Sep	Maize starch, povidone, polyethylene glycol 6000, fumed silica, croscarmellose sodium, talc, magnesium stearate, hypromellose, propylene glycol, and titanium dioxide
	Austral	Metral	L77	10 Feb	
Mexico	Sanofi Aventis	Flagyl	B8B575	11 Mar	
	Limont	Flagenase	P07009	10 Jul	
Peru	Sanofi Aventis	Flagyl	C8R392	11 Jan	
	Hersil	Metronidazole	11017	10 Nov	
	Alkem	Metron	7001EA	10 Mar	
	Genfar	Metronidazol	20108	13 Jan	

Table III. Ziduvudine Products Tested

Country	Company	Product	Batch	Expiry	Excipient
USA	GSK USA	Retrovir	7ZP1642	10 Oct	Corn starch, magnesium stearate, microcrystalline cellulose, and sodium starch glycolate
Mexico	GSK (England)	Retrovir	X5953	05 Oct	
Argentina	Laboratorios Richmonds	Zetrotax	EMX4V	04 Oct	
	Laboratoris Filaxix	Zidovudina	12119D1	06 Oct	Lactose monohydrate, magnesium stearate, microcrystalline cellulose, croscarmellose sodium, and silicon dioxide
	Laboratorio LKM	Crisazet	B853A	04 Oct	Sodium starch glycolate, lactose monohydrate, and magnesium stearate
Uruguay	Laboratorio LKM	Crisazet	B853A	04 Oct	Sodium starch glycolate, lactose monohydrate, and magnesium stearate

a diode-array detector SPD-M10Avp, and data-acquisition software EX Start 7.4 (Shimadzu, Columbia, MS). The mobile phases were degassed before use. The flow rate was 1 mL/min, and the retention time for each drug was about 2 to 2.5 min with a run time of 3 to 3.5 min. Ten-microliter samples were directly injected without dilution.

Amoxicillin

The analytical quantitation of the dissolution samples was modified from the ~~USP~~ monograph for amoxicillin tablets in order to achieve a shorter retention time and better linearity over the expected concentration range of 3.75 to 120-% of labeled content in 900 mL of medium. The HPLC assay used the following conditions: UV detection took place at 219 nm, and the analytical column was an RP 18 LiChrospher 100 column (12.5×4 mm) (Merck, Darmstadt, DE) with guard column. The mobile phase was buffered to pH 5.0 with acetonitrile 5-%. The buffer composition consisted of 6.8 g KH_2PO_4 added to 900 mL of water, after which the pH was adjusted with 45-% (*w*/*w*) KOH to pH 5.0± 0.1 and the volume was filled to 1,000 mL. The method was then tested for suitability with the SIF, buffer pH 4.5, and SGF regarding precision and linearity. The correlation coefficient of the calibration cure was at least 0.999 for each medium, and the percent coefficient of variation were 1.68 in SGF, 1.38 in pH 4.5 buffer, and 1.86 in SIF, respectively.

Metronidazole

The analytical quantification for the dissolution samples was changed from the ~~USP 32~~ procedure. The tablet monograph uses UV absorption at 278 nm for the dissolution test, but the assay uses 254 nm. Metronidazole has another absorption maximum at 228 nm, and this value was used in this study because it resulted in good linearity for drug concentrations between 3.75 and 120-% of the expected drug content in 900 mL of medium. The HPLC assay used the following conditions: UV detection at 228 nm and the analytical column was a Lichrospher RP Select B column (12.5×4 mm) (Merck) with a guard column. The mobile phase was water/acetonitrile (66:34). Analysts validated the modified method for suitability with the media in terms of precision and linearity following procedures in USP general chapter ~~Validation of Compendial Procedures~~ <1225>. The correlation coefficient of the calibration cure was at least 0.999 for each medium, and the percent coefficient of variation were 2.87 in SGF, 0.87 in pH 4.5 buffer, and 2.98 in SIF, respectively.

Zidovudine

The HPLC procedure was modified from that given in ~~USP~~ in order to achieve shorter retention times and used the following conditions: UV detection took place at 265 nm, and the analytical column was a LiChrosphere RP 60 Select B (Merck) with a guard column. The mobile phase was water/acetonitrile: (72:28). The correlation coefficient of the calibration cure was at least 0.999 for each medium, and the percent coefficient of variation were 1.49 in SGF, 2.12 in pH 4.5 buffer, and 2.72 in SIF, respectively.

Study Design

The study design required all equipment and personnel to pass the USP Performance Verification Test (PVT) test in general chapter ~~Dissolution~~ <711>. This criterion is important especially when different labs or multiple personnel or equipment are involved in a study. The PVT ensures that any results generated using standard procedures (whether the studies are conducted in one laboratory or several) comply with the compendial standards established for dissolution test procedures. In this study, all analysts, methods, and equipment passed the PVT test.

Selection of the Comparator Pharmaceutical Product

The preferred CPP according to WHO is an innovator product for which quality, safety, and efficacy has been established in a well-regulated country [*e.g.*, a participant in the International Conference on Harmonization (ICH) or an associated country]. If no innovator product can be identified, an alternative CPP can be chosen. Preferred election criteria are: the CPP has approval in ICH or associated countries; it is "prequalified" by WHO; it has extensive documented use in clinical trials reported in peer-reviewed scientific journals; it has a long and unproblematic period of postmarket surveillance; and finally "well-selected comparators" must conform to compendial quality standards when these exist. The authors used FDA's *Orange ~~b~~ook* to select suitable CPPs (5). When the study was planned, the *Orange ~~b~~ook* listed Amoxil tablets (875 mg amoxicillin tablets from

JrnlID 12248_ArtID 9350_Proof# 1 - 24/03/2012

GlaxoSmithKline) as the RLD (5). There are two different dose-proportional strengths listed in the *Orange ~~b~~ook*, 500 and 875 mg. The WHO list of essential medicines uses the 500-mg strength. However, the RLD was no longer available when the study was performed, and at present, the *Orange ~~b~~ook* lists Amoxil tablets under discontinued products. In order to carry out the study, the authors chose Amoxicillin Sandoz as the CPP because this product was listed in the *Orange ~~b~~ook* as bioequivalent to Amoxil (5). In addition, Sandoz is a global generic manufacturer located in an ICH country as recommended by the WHO guide to identify a well-selected comparator (8). For metronidazol, Flagyl 500-mg tablets (Searle Pharmaceuticals) were the RLD. For zidovudine, Retrovir 100-mg capsules (GlaxoSmithKline) were the RLD. Accordingly, these products were used as CPPs in this study.

Data Analysis

All dissolution data were evaluated using an Excel spreadsheet, and the results were plotted for each product. If the average dissolution of six samples of a drug product at 15 min exceeded 85-% of the labeled drug amount, then no further dissolution tests were performed for this product. If the mean dissolution was below 85-% then six additional units were tested, and a dissolution profile for all 12 samples was generated.

The CPP product was compared with each locally purchased product (test product) according to the following criteria: if both products had >85-% drug dissolution within 15 min (very rapidly dissolving in WHO terminology), they were considered similar in that medium and a profile comparison was not done. Otherwise the products were compared by the f_2 metric. A comparison was also performed between the different test products when appropriate.

In vitro equivalence between test products and CPP and between test products from the same country was established if the dissolution profiles of a test and the comparator product were similar in all three test media according to the f_2 evaluation or if they were considered similar due to very rapid dissolution.

RESULTS

Amoxicillin

The CPP passed the USP Assay test requirements—USP does not require a content uniformity test for amoxicillin tablets (see the amoxicillin monograph and USP general chapter <905>). The weight variation of all tested amoxicillin products showed tablet weights between 676.6 and 752.9 mg. The observed standard deviations for the products ranged between±4.6 and±24.6.

Figure 1 shows the dissolution behavior of amoxicillin products sold in Argentina *vs*. data from the CPP. As seen from the figure, amoxicillin is chemically unstable in SGF, and the drug concentration decreased from the first time point until the end of the observation period.

The CPP, Amoxigrand, and Amoxidal products dissolved rapidly in all three media and were considered to be *in vitro* equivalent. The Telmox, Amixen, and Amox-G products dissolved less than 85-% in 15 min in pH 4.5 buffer and SIF and failed the f_2 comparison criterion with the CPP. Amixen and Amox-G products were similar to each other (f_2=56.6) but neither of them was similar to Telmox (f_2=32.8 and 41.1 for Amixen and Amox-G, respectively). Telmox the Sandoz product sold in Argentina, was not *in vitro* equivalent to the US Sandoz product (500 mg).

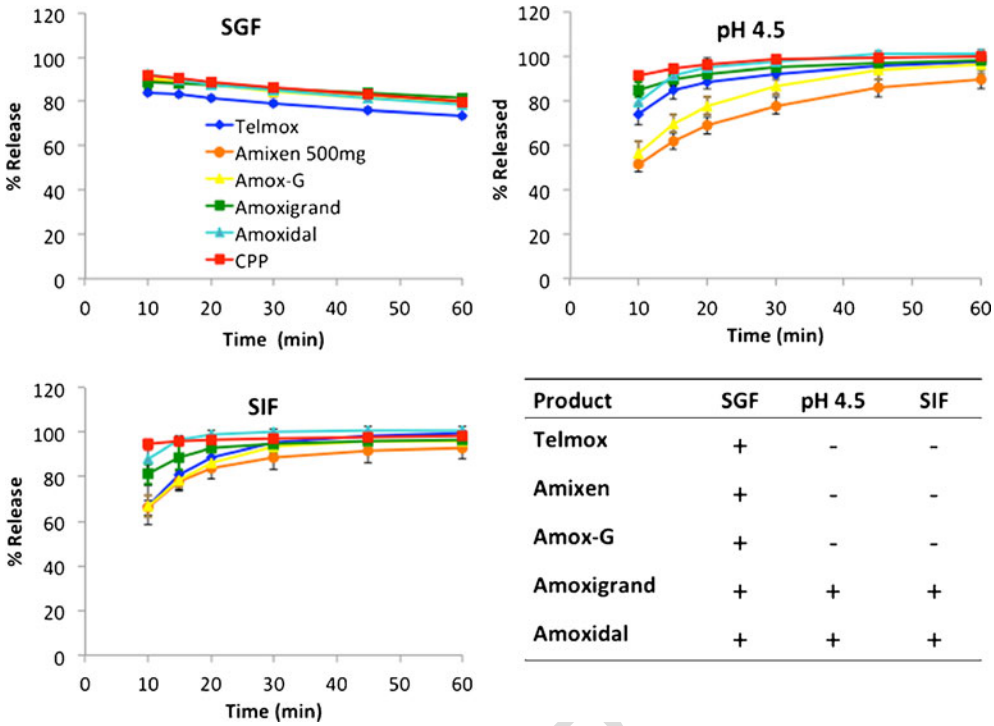
Figure 2 shows the dissolution of products from Chile compared to the CPP. All products dissolved rapidly in SGF. In buffer pH 4.5 the CPP and Amoxicilina product dissolved rapidly, but Amoxicilina LCh, Amobiotic, and Amoval dissolved less than 85 % in 15 min and failed the f_2 comparison with the CPP. However, Amoxicilina LCh, Ambiotic, and Amoval, the f_2 values were similar. In SIF, only the CPP and Amobiotic product dissolved rapidly. The other products dissolved less than 85-% in 15 min, and again Amoxicilina LCh, Ambiotic, and Amoval were not *in vitro* similar to the CPP but the three products had similar f_2 values.

Figure 3 shows the dissolution behavior of products marketed in Peru. The CPP and all generics had similar f_2 values in SGF. Grunamox was found to be *in vitro* equivalent to the CPP. Amoxicilina and Amoval were similar to each other but not to the CPP. Only 3 of 12 tested amoxicillin products showed *in vitro* equivalence to the CPP, and thus only these three can be assumed therapeutically equivalent to the CPP.

Metronidazole

The CPP passed the *USP* assay requirements and the content uniformity test in <905>. The weight variation of all tested metronidazole products showed tablet weights between 697.8 and 771.4 mg. The observed standard deviations for the products ranged between±2.4 and±21.4. Figure 4 shows the dissolution behavior of metronidazole products sold in Argentina *vs*. the CPP. The Flagyl product made by Pharmacia in the USA was the CPP in this study, but Aventis sells their metronidazole product under the same trade name in Argentina and other countries. The Pharmacia and the Aventis products exhibited different dissolution behavior under all test conditions and were not *in vitro* equivalent. In SGF the CPP and the Colpofilin product dissolved rapidly. The other products required more than 15 min to release 85-% of their doses and did not have similar f_2 results compared to the CPP or to each other. In buffer pH 4.5 and SIF, only Ginkan showed similar f_2 results compared to the CPP, and all other products were not similar. None of the four tested products was similar in all three media and therefore no product showed *in vitro* equivalence to the CPP.

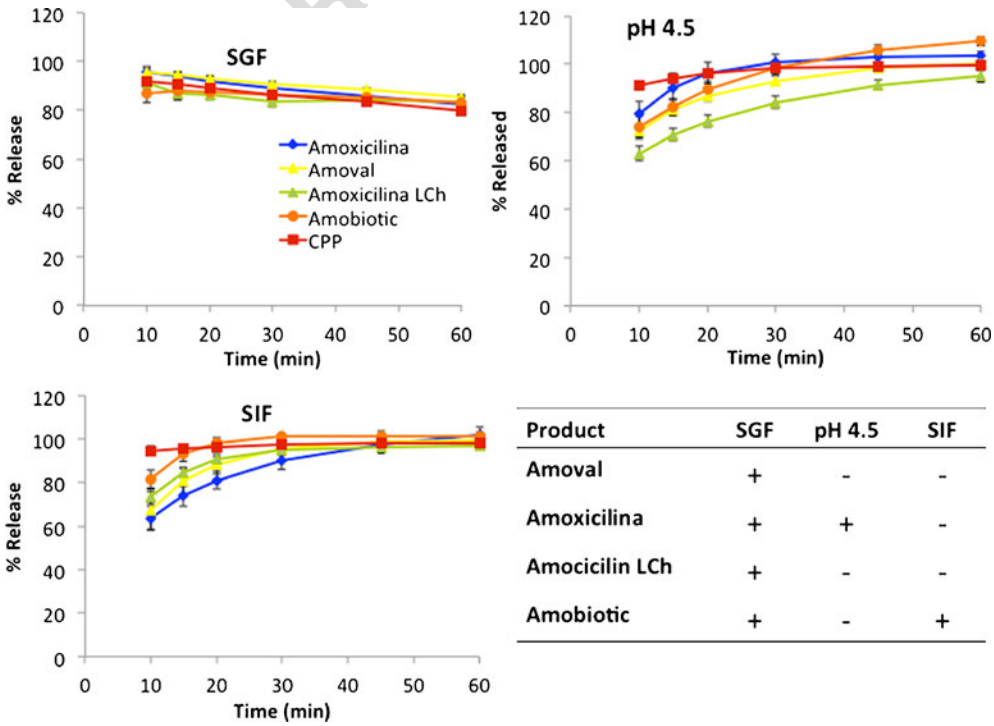
Figure 5 shows the results of the dissolution study of products purchased in Mexico. The CPP dissolved rapidly in SGF. The Flagenase and Flagyl products required 20 and 45 min to release more than 85-% of their doses, respectively. In pH 4.5 buffer, Falgenase dissolved rapidly, but the CPP and Flagyl (Sanofi Aventis) required 30 and 60 min to release more than 85-% of their doses, respectively. In SIF, the CPP and Flagyl required 45 and 60 min, respectively, to release more than 85-% of their contents, but Flagenase dissolved rapidly. None of the tested products showed *in vitro* equivalence to the CPP and did not display *in vitro* equivalence to each other.



Product	SGF	pH 4.5	SIF
Telmox	+	-	-
Amixen	+	-	-
Amox-G	+	-	-
Amoxigrand	+	+	+
Amoxidal	+	+	+

Fig. 1. Dissolution behavior of the CPP and amoxicillin products marketed in Argentina. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium and *negative sign* (−) denotes the lack of similarity

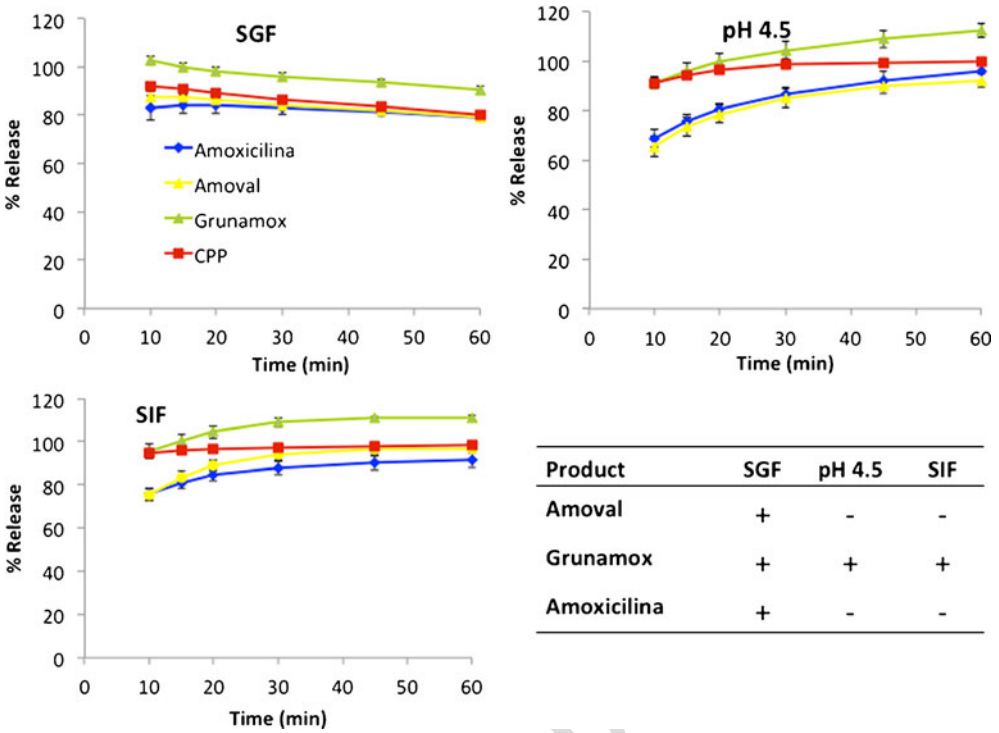
Figure 6 shows the dissolution results from metronidazole products sold in Peru. The CPP, Metron, and Metronidazole Genfar products dissolved rapidly in SGF. In pH 4.5 buffer and SIF, only the metronidazole from Hersil



Product	SGF	pH 4.5	SIF
Amoval	+	-	-
Amoxicilina	+	+	-
Amocicilin LCh	+	-	-
Amobiotic	+	-	+

Fig. 2. Dissolution behavior of the CPP and amoxicillin products marketed in Chile. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium and *negative sign* (−) denotes the lack of similarity

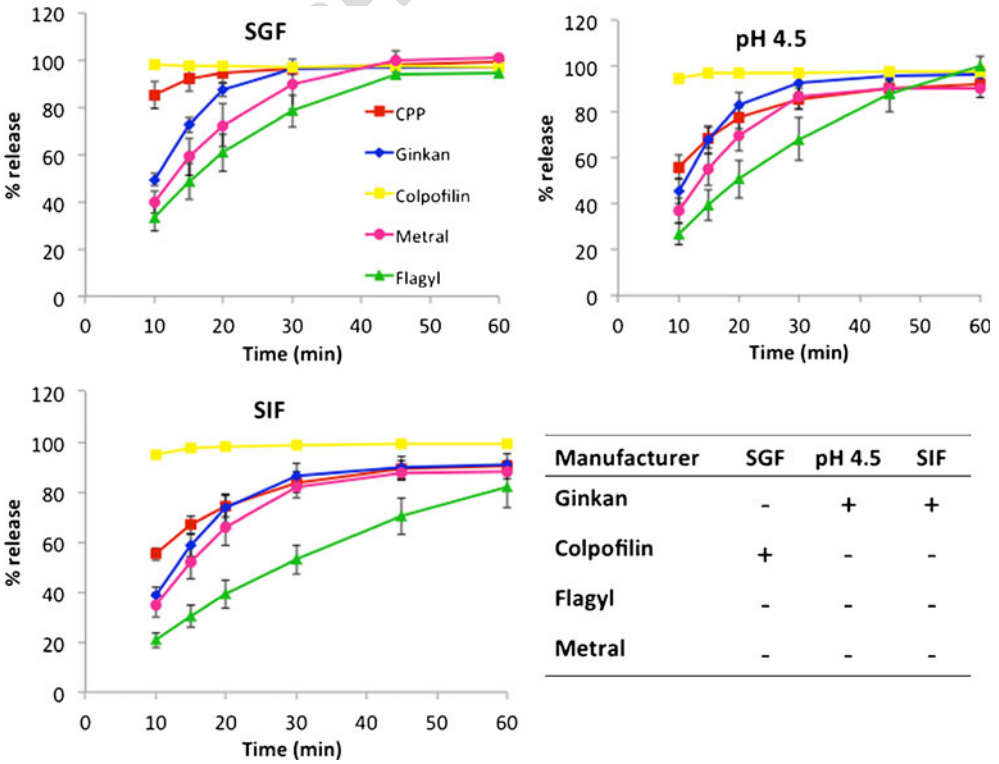
JrnlID 12248_ArtID 9350_Proof# 1 - 24/03/2012



Product	SGF	pH 4.5	SIF
Amoval	+	-	-
Grunamox	+	+	+
Amoxicilina	+	-	-

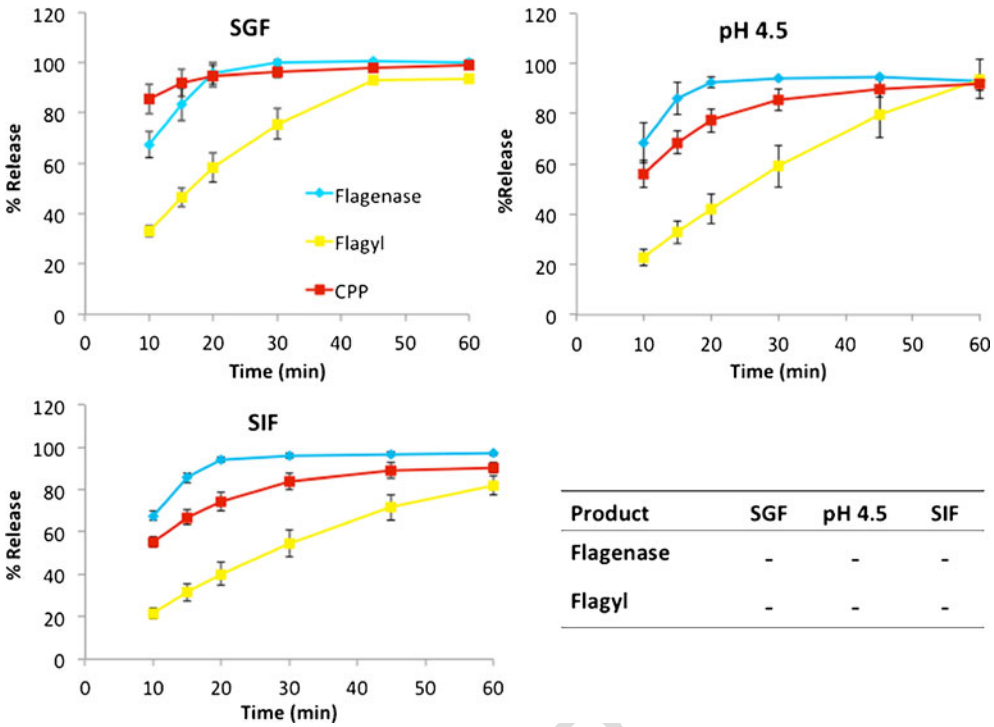
Fig. 3. Dissolution behavior of the CPP and amoxicillin products marketed in Peru. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium and *negative sign* (−) denotes the lack of similarity

showed f_2 values that were similar to those from the CPP. However, this product failed the criteria in SGF and therefore is not equivalent to the CPP. The Flagyl product from Sanofi Aventis had different dissolution behavior compared to the CPP in all media. None of the tested products showed *in vitro* equivalence to the CPP.



Manufacturer	SGF	pH 4.5	SIF
Ginkan	-	+	+
Colpofilin	+	-	-
Flagyl	-	-	-
Metral	-	-	-

Fig. 4. Dissolution behavior of the CPP and metronidazole products marketed in Argentina. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium, and *negative sign* (−) denotes the lack of similarity

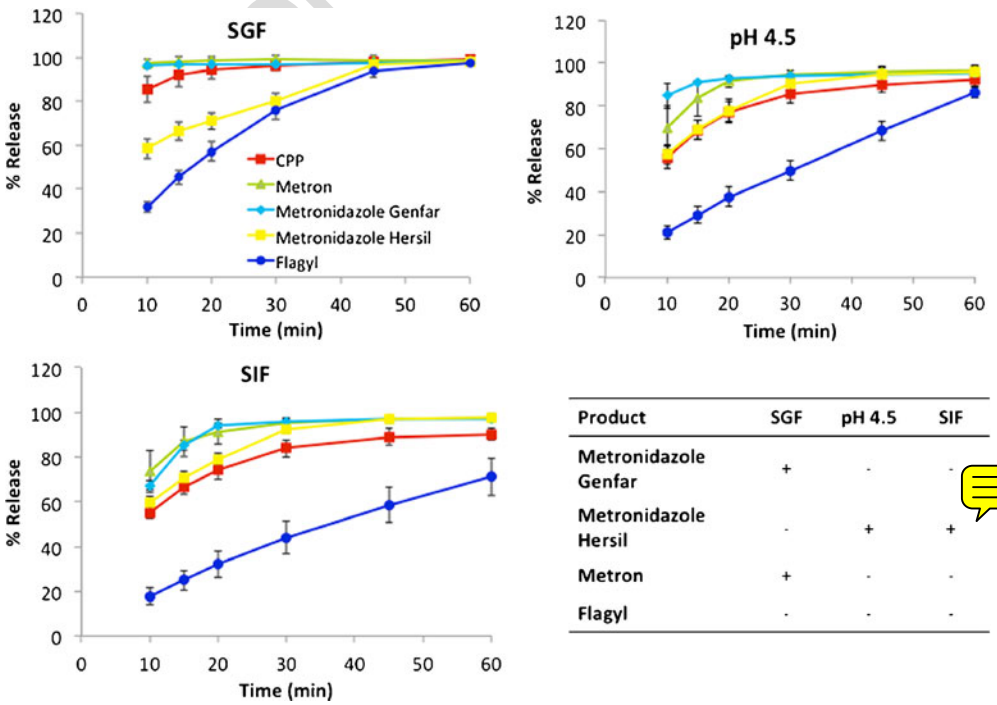


Product	SGF	pH 4.5	SIF
Flagenase	-	-	-
Flagyl	-	-	-

Fig. 5. Dissolution behavior of the CPP and metronidazole products marketed in Mexico. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium and *negative sign* (−) denotes the lack of similarity

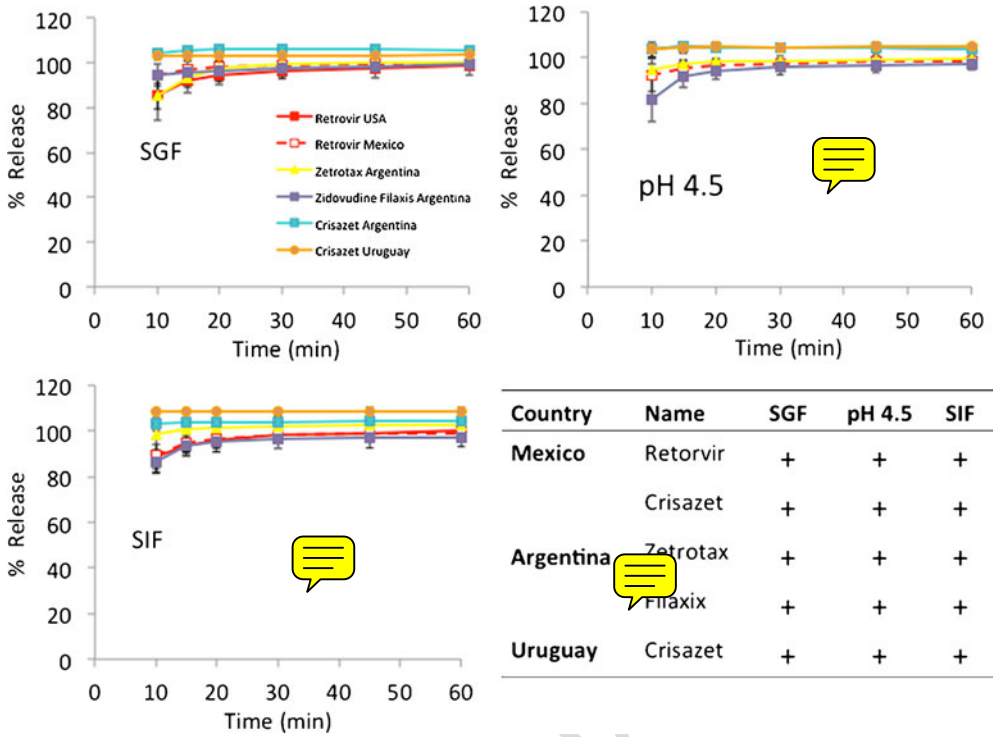
Zidovudine

The CPP complied with USP specification for assay and uniformity of dosage forms. All other products were tested only for weight variation. The weight variation of all tested zidovudine capsules showed average weights between 272.4 and 321.9 mg. The observed standard deviations for the products ranged between±3.1 and±13.6. Figure 7 shows the dissolution behavior of all tested products in all three media. As shown, all investigated products had >85% dissolution



Product	SGF	pH 4.5	SIF
Metronidazole Genfar	+	-	-
Metronidazole Hersil	-	+	+
Metron	+	-	-
Flagyl	-	-	-

Fig. 6. Dissolution behavior of the CPP and metronidazole products marketed in Peru. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium and *negative sign* (−) denotes the lack of similarity



Country	Name	SGF	pH 4.5	SIF
Mexico	Retorvir	+	+	+
	Crisazet	+	+	+
Argentina	Zetrotax	+	+	+
	Filaxix	+	+	+
Uruguay	Crisazet	+	+	+

Fig. 7. Dissolution behavior of the CPP and zidovudine products marketed in the Americas. The *table* summarizes the comparison between the CPP and the different products: *positive sign* (+) denotes similarity with the CPP in the specified medium and *negative sign* (−) denotes the lack of similarity

within 15 min. All products show *in vitro* equivalence according to the WHO guideline. They can be considered as therapeutically equivalent. The Retrovir products purchased in the USA and Mexico had superimposable dissolution behaviors in SIF.

DISCUSSION

The study showed the challenges of identifying a CPP when the original RLD is no longer available (18). In this case, the originally listed amoxicillin RLD from the *Orange book* was withdrawn from the market while the study was planned, and the *Orange book* had not defined a replacement RLD. The researchers selected a CPP using the WHO criteria, as mentioned above. While the study was in progress TEVA's generic product was identified in the *Orange book* as the US replacement RLD. Challenges to obtain certain products were observed for individual countries too. For example, GlaxoSmithKline Peru S.A. marketed Amoxil 12 H in Peru, but this amoxicillin product was not commercially available when the study was undertaken. Thus the authors were unable to determine if this product is identical to the US product. GSK did not market amoxicillin tablets in other countries that were included in this study. These cases demonstrate how difficult it can be to identify an appropriate CPP for each country. Furthermore, Sandoz's amoxicillin 500 mg product sold in Argentina did not show *in vitro* equivalence to Sandoz's US product, which was chosen as the CPP. The excipient content list (Table I) shows that these two products were formulated differently. Sandoz clarified the difference by explaining that "amoxicillin tablets marketed in Argentina were developed as generic medical products for the European Union (EU) market based on the company's bioequivalence study CPA 45/97. In this study, the bioavailability of the generic medicinal product OSPAMOX 750 mg FCT, batch 95362 (Biochemie GmbH, Austria) was compared with the reference medicinal product Clamoxyl 750-mg tablets, batch 96D15/32335 (SmithKline-Beechem Pharma GmbH, Germany). Because the 90% confidence intervals for the primary bioequivalence parameters were within the prespecified limits of 80–125%, the study demonstrated the bioequivalence of the tested formulations" (Sandoz, personal communication, 2010).

The Sandoz product sold in Argentina was developed in Europe and its BE was tested against a European product that has a different strength compared to the US innovator product (Amoxil GSK). This does not imply that these products are substandard but rather that they were developed to match a different CPP. This study shows that different products from different countries may have different *in vitro* dissolution even if they contain the same drug and strength and are made by the same manufacturer in the same facility. Importantly, this kind of information typically is not publicly available. Except for the Sandoz product, the authors do not know if the other generics tested underwent bioequivalence testing and which CPP was used. This complicates a comparison of amoxicillin products across different countries. The data give a good overview of *in vitro* product performances, but any comparisons among them must be limited to the *in vitro* results.

If a product did not show *in vitro* equivalence to the CPP, the product is not necessarily bioinequivalent. Its bioequivalence could have been documented using one of the several *in vivo* options. The study results showed that selected products are available and that they demonstrate *in vitro* equivalence to the chosen CPP. This is particularly important because the CPP

used in this study presumably was not developed for all climate zones according to ICH (19).

In the case of metronidazole, the study found that two different products with the same trade name, Flagyl, are marketed in the Americas. The CPP is from G.D. Searle LLC, which is a Pharmacia subsidiary, which in turn is owned by Pfizer. The Sanofi Aventis Flagyl showed different dissolution behavior in all media compared to the Pharmacia product and may not be therapeutically equivalent. The comparison of the Sanofi Aventis products procured in different countries showed that dissolution profiles of the products from Peru and Argentina were similar in SIF and SGF but not in buffer pH 4.5 (f_2=43.4; graph not shown). These differences were not linked to the differences in their expiration dates (see Table II). All three batches were produced in the same factory as stated on the packages and were imported from Mexico to Argentina and Peru. This suggests a more general question about how many batches of a CPP should be investigated before it can be used as CPP in a biowaiver study. There is currently no requirement by any FDA, European, or WHO ~~B~~ioequivalence guidance document to investigate different batches for *in vivo* bioequivalence studies. These results suggest another question: Can a CPP be used for a biowaiver study if three batches were found not to have *in vitro* equivalence?

The study hypothesis was confirmed only for the zidovudine products, which showed *in vitro* equivalence to each other and the CPP. Supplemental Fig. 1 shows two GSK Retrovir products manufactured in the US and England (purchased in Mexico). The product manufactured in England has a seal between the cap and the capsule body (blue strip). The seal is necessary because of the products' different packaging. The blister pack of the US product must be peeled open at the edges to dispense the capsule, but the sealed capsule of the product made in England must be forced through the aluminum foil of the blister. If the US product is forced through the back liner of its blister, the capsule might dent or break with spillage of contents because of the tensile strength of the back foil. Because the product made in England is exposed to higher forces when it is pressed through the back liner of its blister, the capsule's cap and body must be sealed to prevent spilling. This shows that different regions in the world may require different packaging for the same product, and this can cause adjustments in the dosage forms, as seen for Retrovir. However, as seen from the dissolution profiles for these products, the additional seal did not influence the *in vitro* performance of the product.

Supplemental Fig. 2 shows a blister pack of a generic product available in Arg and Uruguay. The capsules were not manufactured properly, and some drug spilled out of the capsules. Several blisters of this product contained one or two capsules that showed this defect. None of the defect capsules were used for the dissolution study. During manufacturing and packaging, visual quality control should have removed such blisters before batch release. Another observation is that these capsules use the same type of blister as the Retrovir capsules made in England. However, these capsules have no seal between capsule body and cap to avoid content spill when the capsules are pressed through the blister. The aluminum foils were determined to be 0.04 mm for the Retrovir blister and 0.03 mm for the generic, which might explain the addition of the seal between cap and body when a thicker blister foil is used.

CONCLUSIONS

All tested zidovudine products showed *in vitro* equivalence to each other and the CPP. Only 3 of 12 tested amoxicillin products showed *in vitro* equivalence to the CPP. None of the tested metronidazole products exhibited *in vitro* equivalence to the CPP. Two different metronidazole products with the same trade name are marketed globally. These products have different biopharmaceutical properties and were not *in vitro* equivalent.

As advocated by WHO and others, the issues and challenges in identifying a CPP in different countries clearly suggest the potential value for establishing an international reference standard product to support bioequivalence studies. Working with such a product, the generic industry in developing countries could use an internationally accepted reference standard to develop therapeutically equivalent and thus interchangeable multisource products. Innovator manufacturers would also be able to use such a product to compare selected formulations. At this time, clinicians should generally avoid assumptions that formulations sold across national boundaries are therapeutically equivalent, even when labeled to contain the same drug substance and strength.

ACKNOWLEDGMENT

The authors thank Stefan Schuber, PhD, ELS, at USP for editorial assistance with this manuscript.

REFERENCES

1. World Health Organization. Essential medicines and pharmaceutical policies. 2010. http://www.who.int/medicines/en/. Accessed 14 Nov 2011.
2. Seear M, Gandhi D, Carr R, Dayal A, Raghavan D, Sharma N. J Clin Pharm Ther. 2011;36(4):488–95. doi:10.1111/j.1365-2710.2010.01198.x.
3. Caudron JM, Ford N, Henkens M, Macé C, Kiddle-Monroe R, Pinel J. Substandard medicines in resource-poor settings: a problem that can no longer be ignored. Trop Med Int Health. 2008;3(8):1062–72.
4. Van Roey J, Haxaire M. The need to reform current drug registration processes to improve access to essential medicines in developing countries. Pharm Med. 2008;22(4):207–13.
5. FDA. Orange book. 2010. http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm. Accessed 14 Nov 2011.
6. WHO. Fortieth report, annex 8: proposal to waive *in vivo* bioequivalence requirements for WHO model list of essential medicines—immediate-release, solid oral dosage forms. Geneva: WHO; 2006. p. 391–438.
7. Lobenberg R, Amidon GL. Modern bioavailability, bioequivalence, and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur J Pharm Biopharm. 2000;50(1):3–12.
8. Blume HH, Schug BS. The biopharmaceutics classification system (BCS): class III drugs—better candidates for BA/BE waiver? Eur J Pharm Sci. 1999;9(2):117–21.
9. Benet LZ, Larregieu CA. The FDA should eliminate the ambiguities in the current BCS biowaiver guidance and make

JrnlID 12248_ArtID 9350_Proof# 1 - 24/03/2012

public the drugs for which BCS biowaivers have been granted. Clin Pharm Ther. 2010;88(3):405–7.

10. CDER. Guidance for industry: waiver of *in vivo* bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. 2000. www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070246.pdf. Accessed 14 Nov 2011.
11. European Medicines Agency. Guideline on the investigation of bioequivalence. 2010. http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500070039&mid=WC0b01ac058009a3dc. Accessed 14 Nov 2011.
12. Williams RL, Chen M-L, Hauck WW. Equivalence approaches. Clin Pharm Ther. 2002;72(3):229–37. doi:10.1067/mcp.2002.126705.
13. WHO. Fortieth report: annex 7: multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. Geneva: WHO; 2006. p. 347–90.
14. Blume H, Schug B. Bioavailability/bioeqivalence requirements of immediate release products: resolutions and issues. In: Midha K, Nagai T, editors. Bioavailability, bioequivalence, and pharmacokinetic studies. Tokyo: Business Centre for Academic Societies Japan; 1996. p. 85–90.
15. Blume H, Ali SL, Siewert M. Pharmaceutical quality of glibenclamide products: a multinational postmarket comparative study. Drug Dev Ind Pharm. 1993;19(20):2713–41. doi:10.3109/03639049309050174.
16. Horne C, Stenzhorn G, Blume H, Knauf H, Mutschler E. Bioavailability study of two different verapamil formulations. Arch Pharm. 1992;325(8):531–6.
17. USP. Compendial tools. 2009. http://www.usp.org/USPNF/compendialTools.html. Accessed 14 Nov 2011.
18. Williams RL, Shah VP. Continuing equivalence: is there an end to the story? J Generic Med. 2008;5(4):297–304.
19. ICH. Q1A(R2): stability testing of new drug substances and products. 2003. http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html. Accessed 14 Nov 2011.