Does GastroPlus Support Similarity and Dissimilarity Factors of in vitro-in vivo Prediction in Biowaiver Studies? A Lower Strength Amlodipine As a Model Drug

Authors
Abdel Naser Zaid, Naser Shraim, Asmaa Radwan, Nidal Jaradat, Samah Hirzallah, Ibrahim Issa, Aya Khraim

Affiliation
Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus - Palestine

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Correspondence
Professor Abdel Naser Zaid
Director of Pharmacy Department
Faculty of Medicine & Health Sciences
An-Najah National University Nablus P.O. Box 7
Palestine
Tel.: +970/972/92345 113, Fax: +970/292/345 982
anzaid@najah.edu

ABSTRACT

Background Many generic pharmaceutical products are currently available on the market place worldwide. Recently, there is a growing concern on the quality and efficacy of generic products. However, health care professionals such as physicians and pharmacists are in difficult situations to choose among alternatives.

Purpose The aim of this study is to assess the effectiveness of the in silico technique (Gastro Plus®) in the biowaiver study and whether similarity and dissimilarity factors (f_2 and f_1 respectively) are effective in this regard.

Method The concentration of amlodipine in the sample was calculated by comparing the absorbance of the sample with that of a previously prepared amlodipine standard solution using validated HPLC method. The dissolution profile for each product (brand and generics) was constructed. The similarity (f_2) and dissimilarity (f_1) factors were calculated for the generic product according to equation 1 and 2. GastroPlus™ software (version 9.0, Simulations Plus Inc., Lancaster, CA, USA) was used to predict the absorption profiles of amlodipine from the generic product Amlovasc® and the reference Norvasc®.

Conclusion These results may provide a rationale for the interchangeability between the RLD and generic version based on in vitro release profiles in silico technique especially in a lower strength dose drug.

Introduction

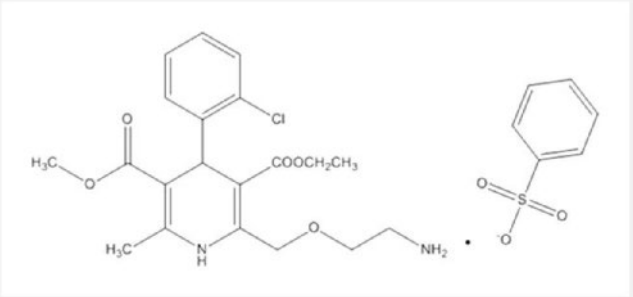
Many generic versions of pharmaceutical products are currently available on the marketplace worldwide. Recently, there has been a growing concerns on the quality and efficacy of these generic products. However, health care professionals such as physicians and pharmacists are in difficult situations to choose among alternatives, since they usually do not trust new generic products [1]. The bioavailability of the selected drug from different oral formulations is an important aspect that can be used to compare safety and efficacy issues of these formulations. Generics might lack bioavailability similarity compared to the reference listed drug (RLD) and consequently efficacy and safety concerns become questionable [2].

Since 1960 s, in vivo pharmacokinetic bioequivalence (BE) studies have emerged as “gold standards” in proving similarity and interchangeability between innovator products and their generic versions. BE studies entail comparing the plasma or urine concentration versus time profiles of a test versus a RLD product. Unfortunately, this tool is costly and time consuming, since it involves invasive tests on humans [2–4]. Recently, in vitro dissolution testing has emerged as powerful tool in predicting in vivo bioavailability of oral drug formulations. The advent of the biopharmaceutical classification system (BCS) and the wide adoption by the regulatory agencies around the globe, especially the United States Food and Drug Administration (FDA), European Medicines Agency (EMA) and the World Health Organization (WHO) have fundamentally changed the drug approval process for immediate release (IR) solid oral formulations [3, 5]. Regulatory agencies now can waive the in vivo BE studies for IR solid oral formulations containing high solubility (BCS class I and III) drugs and grant the formulation with a marketing authorization based on a biowaiver application [5, 6]. Based on the biowaiver principles, very rapid dissolution or rapid

dissolution with similarity factors are enough proof of similarity if the drug has a wide therapeutic index and the formulation contained non-interfering excipients [3, 5]. The Focus Group on BCS and biowaivers of the International Pharmaceutical Federation (FIP) has invited scientists around the world to prepare biowaiver monographs evaluating the suitability of waiving in vivo BE studies for drugs listed on the WHO's essential medicines list [7, 8].

In silico modelling has been proven to be useful in predicting the in vivo performance of drugs. Gastrplus simulation has many applications in the drug development process, including: the prediction of bio equivelance and justification of a biowaiver. In silico techniques were implemented to predict the in vivo absorption profiles and the bioequivalence of some BCS class I and III drugs and to assess the feasibility of extending biowaivers to these compounds [9]. Okumo et al. (2009) [10] used gastrointestinal simulation technology to aid the justification of a biowaiver for eterocoxib from solid oral dosage forms. Similarly, Kovačević et al. [11] utilized GastroPlus simulations to investigate a possible extension of a biowaiver for BCS II drug, Carbamazepine. Amlodipine [12] (IUPAC Name: 2-[(2-Aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl 5-methyl ester benzenesulfonate) (▶ **Fig. 1**). ▶ **Table 1** has summarized the most important physicochemical properties of amlodipine.

Amlodipine is a potent peripheral and coronary vasodilator with high selectivity for vascular smooth muscles with lower effect on myocardial contractility or cardiac conduction [14]. It works by inhibiting transmembrane influx of extracellular calcium ions across membranes of myocardial cells and vascular smooth muscle cells without changing serum calcium concentrations; this inhibits cardiac and vascular smooth muscle contraction, thereby dilating main coronary and systemic arteries, it also Increases myocardial oxygen delivery in patients with vasospastic angina [15]. Amlodipine is indicated for hypertensive patients as a blood pressure Lowering drug, thus it will reduce the risks of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions [16]. Due to its importance in the treatment of blood hypertension, amlodipine is listed in the WHO's List of Essential Medicines [17].



▶ **Fig. 1** Chemical structure of amlodipine.

▶ **Table 1** Physicochemical properties of amlodipine [13].

Molecular formula	$C_{20}H_{25}ClN_2O_5$
Molecular weight	408.88 g/mole
Exact mass	408.1452 g/mole
Melting point	178–179 °C
Solubility	Water Solubility (75.3 mg/L)
Vapor pressure	1.19×10^{-9} mm Hg at 25 °C
Log P	log $K_{o/w} = 3.00$
pKa	8.6 at 25 °C

Amlodipine undergoes gradual absorption from the gastrointestinal tract with an oral bioavailability ranging from 64 % to 90 %. It is widely distributed throughout the body tissues [18]. The maximum plasma concentration of amlodipine can be obtained within 6–12 h [15]. It is cleared only slowly by metabolism in the liver and so has a long elimination half-life ranges from 30 to 50 h [18]. The major metabolite is 2-([4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-pyridyl]methoxy)[19]. It is classified as high permeable drug due to metabolite excretion in urine (90–95 %) [20]. Adverse reactions may occur during the course of this medicine, for example: headache, swelling of legs or ankles, tiredness, extreme sleepiness, stomach pain, nausea, dizziness, flushing, arrhythmia. Amlodipine has been assigned to pregnancy category C by the FDA, so it should be used during pregnancy only if the potential benefit justifies the risk to the fetus. It is recommended that amlodipine must be discontinued during breastfeeding [21].

In this study, we attempt to assess the effectiveness of the in silico technique (Gastro Plus®) in the biowaiver study and whether similarity and dissimilarity factors (f_2 and f_1 respectively) are effective in this regard. In addition, these results may provide a rationale for the interchangeability between the RLD and generic version based on in vitro release profiles in silico technique especially in a lower strength dose drug.

Materials, Instruments & Methods

Chemicals & reagents

Potassium dihydrogen orthophosphate, and hydrochloric acid (37 %) were purchased from Sigma-Aldrich (Germany), sodium hydroxide and orthophosphoric acid were purchased from Merck KGaA (Germany). Menthol from Carlo Erba reagents (Italy). Purified water was obtained using a Millipore mille-Q water purification system (Conductivity between 0.9-1.2; pH between 5.6-5.9). Amlodipine besylate standard (Switzerland) and the tested products Norvasc® 5 mg/tablet (Pfizer) Amlovasc® 5 mg/tablet (Pharmacare Ramallah, Palestine) were purchased from a community pharmacy shop (Ramallah, Palestine).

Instruments

A double beam UV/Visible spectrophotometer (Jenway 7305- Tron, UK) was used for the quantitative analysis of amlodipine. Automated dissolution equipment (Pharma test, Germany) was used to assess the dissolution behavior and release of the active ingredient from tablets. Balance Ohaus (Discovery, Switzerland) for weighing, Centrifuge (Hermal, Germany). The pH of the dissolution media was adjusted with a MP230 pH meter (Metter Toledo, Switzerland). Ultrasonic Cleaner (Branson, Mexico) was used to accelerate the dissolution of amlodipine powder, Magnetic stirrer (Velp scientific, Europe), Disposable syringe (Medi-puls, China), laboratory glassware (Volumetric flasks, measuring cylinder's, volumetric pipettes and graduated pipettes) were supplied by Pharmacare PLC.

Methods

The general appearance of all tested tablets was visually examined. Each selected tablet of all generic and brand products was weighed using highly sensitive electronic balance (Ohaus) and the weights were registered. In vitro, dissolution studies were conducted in order to compare the dissolution rate of the generic with the brand. The test was carried out using type II dissolution apparatus according to the USP. Paddles covered with Teflon were used to avoid any incompatibilities between amlodipine and stainless-steel. Three kind of dissolution medium, pH 1.2, 4.5, 6.8, were used in this study. A volume of 900 mL of each media was placed in each paddle and the solution was kept at 37 ± 0.5 °C during the entire period of the dissolution study. One tablet was placed in each one of the dissolution vessels. The dissolution media was kept under 50 rpm mixing speed. Samples of 10 mL were taken at the following time intervals 10, 20 and 30 min. The taken volumes were replaced by same volume of blank dissolution medium. Each sample was analyzed using the spectrophotometric method, amlodipine was detected at wavelength 237 nm according to conditions described in the USP [22]. The concentration of amlodipine in the sample was calculated by comparing the absorbance of the sample with that of a previously prepared amlodipine standard solution. The dissolution profile for each product was constructed. The similarity (f_2) and dissimilarity (f_1) factors were calculated for the generic product according to ▶ **Eqs. 1**, **2** [12, 23, 24].

$$f_1 = \left\{\left[\sum_{t=1}^{n}\left|R_t - T_t\right|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} \times 100 \quad (1)$$

$$f_2 = 50 \cdot \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}\left(R_t - T_t\right)^2\right]^{-0.5} \times 100\right\} \quad (2)$$

f_1 stands for dissimilarity while f_2 stands for similarity factors

Gastrointestinal simulation

GastroPlus™ software (version 9.0, Simulations Plus Inc., Lancaster, CA, USA) was used to predict the absorption profiles of amlodipine from the generic product Amlovasc® and the reference Norvasc®. The software, which is based on the Advanced Compartmental Absorption and Transit (ACAT) model, consists of three modules: compound, physiology, and pharmacokinetics. For the compound and pharmacokinetics modules; the input data were either determined experimentally, or taken from the literature. In the physiology module, the human physiology under the fast state mode was selected and the default values were used. The in vitro dissolution data for the drug in the different media were used as input functions in Gastroplus™ using the controlled release-dispersed dosage form (CR-dispersed) and the "tabulated in vitro dissolution data" functions. The simulations were carried out using an immediate release (IR) tablet as the selected dosage form. The absorption profiles were compared using the dissolution profiles. The summary of all input parameters for simulation is given in ▶ **Table 2**.

The percent of prediction error of the simulation was calculated using the following equation:

▶ **Table 2** Simulation input data.

Parameter	Amlodipine
Molecular weight (g/mole)	567.051
Partition coefficient	2.66 (pH = 7.4) [a]
Pka_1	8.7 [b]
Solubility (mg/ml)	0.774 (pH 7.4) [c]
P_{eff} (Human jejunal permeability) (cm/sec)	0.0743 * 10^{-4} [d] (caco-2)
Dose (mg)	5
Dose volume (ml)	250
Mean precipitation time (sec)	900 [e]
Diffusion coefficient (cm^2/s)	4.2 * 10^{-8} [g]
Drug particle density (g/ml)	1.2 [e]
Blood plasma concentration ratio	1 [e]
Body weight (kg)	70
Unbound percent in plasma (%)	2 [f]
Clearance (l/hr.)	28 [h]
Volume of distribution, Vc (L/Kg)	17 [h]
Elimination half-life (h)	27.03 [I]
Simulation time (hr)	144

[a] From [25, 26]; [b] From [27, 28]; [c] From [29]; [d] From [26]; [e] From Gastro Plus default values; [f] From [31]; [g] From [30]; [h] Gastro Plus calculated (using PBPKPlus™ Module); [I] Gastro Plus calculated (built-in calculation from PK parameters)

$$\%PE = \{(PK_{predicted} - PK_{observed})/PK_{observed}\} * 100\%$$

Results

All tested products were inspected for visual appearance, no sign of defects or abnormalities were observed. Regarding the weight uniformity of the tested tablets, both RLD and generic were within the recommended weight uniformity range, as reported in the USP. Precisely, Norvasc® average weight was 0.201092 ± 1 %, while Amlovasc® average weight was 0.2000 ± 5 % respectively. Concerning the release of AM from generics and RLD tablets, the two tablet products showed comparable dissolution behavior in all three recommended pH media for similarity studies. The release of amlodipine from Amlovasc® (5 mg/ tablet) in the previously mentioned dissolution media is reported in ▶ **Table 2**. The results show that the release of amlodipine was comparable with the original brand (Norvasc® 5 mg/ tablet) since f_2 and f_1 were higher than 50 and less than 15 respectively as shown in ▶ **Table 3** and **4** (▶ **Fig. 2–4**).

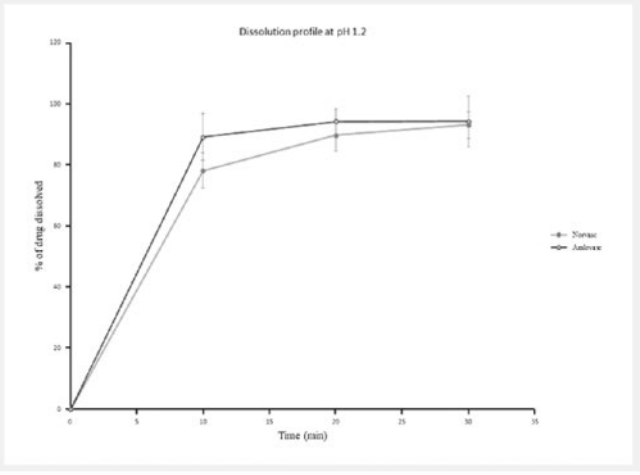
All tested products were found to contain the same content of inactive pharmaceutical ingredient, as reported in ▶ **Table 5** [32].

In silico Simulation

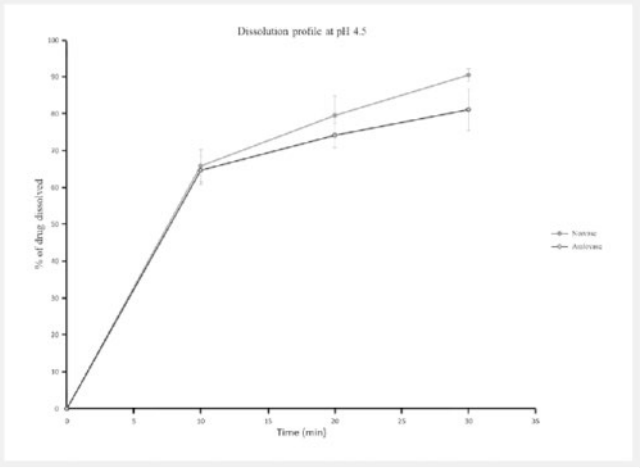
GastroPlus™ was used to simulate the absorption profile of the generic Amlovasc® and the reference drugs Norvasc® in order to check the bioequivalence of the test formulation. Computer simulations were performed using the dissolution data in the different media (in 0.1 N HCl, Phosphate buffer pH 4.5 and 6.8.). ▶ **Fig. 5**, **6** show the observed and simulated plasma profiles for the generic and the brand. The simulated profiles were similar and superimposable indicating that there is no difference between the oral absorption the

▶ **Table 3** Release of amlodipine from Amlovasc® and Norvasc®.

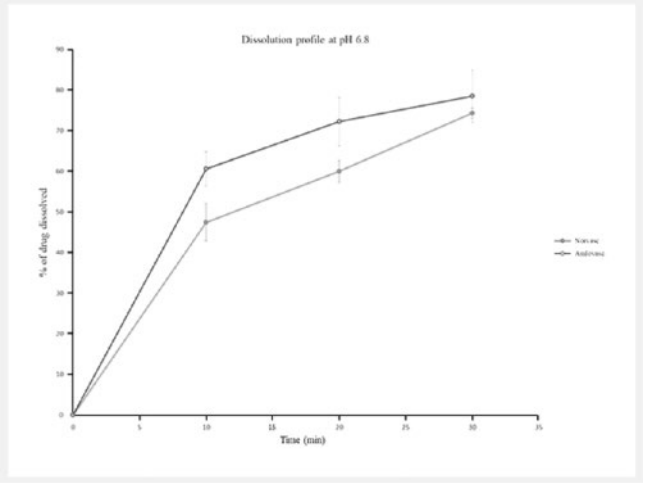
Time (Minutes)	pH = 1.2		pH = 4.5		pH = 6.8	
	Norvasc ± SD	Amlovasc ± SD	Norvasc ± SD	Amlovasc ± SD	Norvasc ± SD	Amlovasc ± SD
0	0	0	0	0	0	0
10	78.2 ± 5.7	89.2 ± 7.6	65.9 ± 4.4	64.7 ± 3.8	47.4 ± 4.6	60.6 ± 4.3
20	89.8 ± 5.3	94.2 ± 4.3	79.6 ± 5.3	74.2 ± 3.3	60.0 ± 2.7	72.2 ± 6.0
30	93.1 ± 4.3	94.3 ± 8.3	90.6 ± 1.7	81.2 ± 5.7	74.3 ± 1.3	78.5 ± 6.5



▶ **Fig. 2** Dissolution profile at pH 1.2 (batch 1).



▶ **Fig. 3** Dissolution profile at pH 4.5 (batch 1).



▶ **Fig. 4** Dissolution profile at pH 6.8 (batch 1).

▶ **Table 4** Results of similarity and non-similarity factors for Amlovasc®.

Product name	pH1.2		pH4.5		pH6.8	
	F2	F1	F2	F1	F2	F1
Amlovasc® (Batch No.1)	68.2	4	69.9	1	59	7

two products in the different pH. Furthermore, the observed values obtained for Cmax and AUC were close to the simulated values. The prediction errors were less than 10 %. ▶ **Table 6** compares the AUC and Cmax values for the reference and the generic.

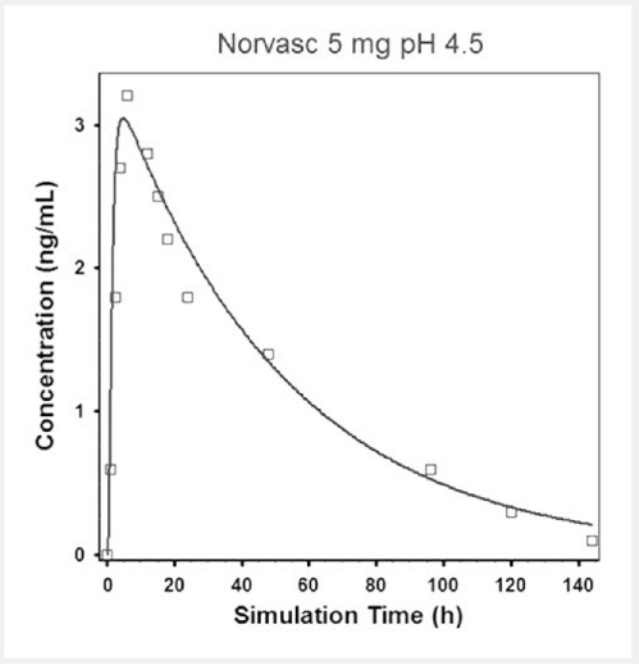
Discussion

According to the WHO list of essential drugs, AM (5 mg tablet) was classified as class I product, since it showed a high therapeutic index, high permeability, and solubility. Related to this classification any pharmaceutical company can use biowaiver criteria in pre-marketing pharmaco-vigilance. This study can also be submitted to regulatory authorities in order to register the generic products without the need of BE studies. On condition that both API & formulation satisfy the biowaiver criteria. Accordingly, the pharmaceutical company should submit all data that support their file application in order to convince the regulatory authorities about the safety and efficacy of this abbreviated new drug application.

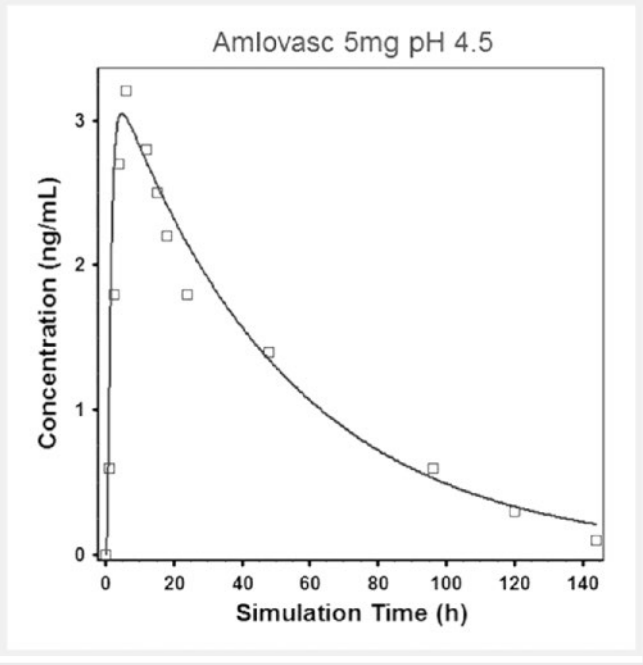
Therefore, the aim of this study was to prepare a biowaiver monograph based on both literature data and in vitro testing of AM as the only API present in tablet products. This kind of studies is based on the BCS properties and the risk of waiving in vivo studies of the API.

The risk is defined as the probability of an unsuitable biowaiver decisions in terms of public health and individual patient risks. In respect to these considerations, a recommendation can be made to whether a biowaiver approval is desirable or not.

According to the collected literature data, AM was considered as a safe drug and exhibits a low potential for acute toxicity. In fact, the lethal dose (LD_{50}) values were around 37 mg/kg for mice, 393 mg/kg for rats (USP, 2006). Moreover, the dosage regimen can vary from 2.5 mg daily to 10 mg daily. The most common adverse reactions in humans are dizziness, nausea, edema, stomach pain & palpitation [32].



▶ **Fig. 5** In silico predicted and in vivo observed pharmacokinetics for Norvasc.



▶ **Fig. 6** In silico predicted and in vivo observed pharmacokinetics for Amlovasc.

Other properties, consider important in biowaiver, were also evaluated for AM. In fact AM absolute bioavailability is 60–65 %, however, its permeability classified as high because of its metabolite that excreted in urine (90–95 %), according to USP, when an API is absorbed to 85 % and more, its considered " highly permeable" [33]. AM is described as slightly soluble in water (USP, 2006). The water solubility for AM is 75.3 mg/L (Drug bank database, 2010). The lowest solubility in the pH range from 1 to 6.8 at 37 °C is 1 mg/mL (WHO, 2006). Thus the Dose/Solubility ratio for AM WHO Model List of Essential Medicines dose (5 mg) at a pH range of 1.2–6.8 is 5 mL and 10 mL for the highest dose. Therefore, AM is a "highly soluble" drug according to WHO Guidance (D/S ratio ≤ 250 mL).

Interchangeability between a generic and brand is a common pharmacy practice. However, the generic product should resemble as much as can the general appearance and in vitro quality of the origin brand. The evaluated generic product showed visual appearance, size, shape and weight comparable to the original brand. However, the most crucial quality to be considered in biowaiver monograph & for a successful interchangeability is the dissolution profiles of the generic according to the international guidelines for biowaiver studies. The results of dissolution showed that both generic products were comparable with the original brand. In fact, there was no need to calculate f_1 and f_2 at pH 1.2 since the release was higher than 85 % even after 10 min. Moreover, f_2 and f_1 were within the recommended criteria of biowaiver studies for Vascopin and Amlovasc at pH 4.5 and 6.8.

Furthermore, another batch of the original brand was analyzed for dissolution behavior and both generics were compared with again. f_2 and f_1 were higher than 50 and less than 15 also in this second batch. Regarding the excipients content of generic and brand, biowaiver criteria consist the use of same excipient, a very important factor for the correct decision, since some excipients may influence the intestinal permeability. In fact, all studied products were found to contain the same API. The comparison of the simulated profiles generated using dissolution data in the different pH media indicates that the generic has similar in vivo behavior to the brand. This is due to the rapid dissolution and drug permeation (BCS class 1). According to the FDA's guidance on Bioequivalence Studies, two products are considered bioequivalent if the 90 % confidence interval (CI) of the relative mean Cmax, AUC of the generic to the reference formulation should be within 80 % to 125 % in the fasting state. In this study, the bioequivalence calculations show the similarities between AUC and C_{max} for the Amlovasc versus the Norvasc.

The percent of drug dissolved was more than 85 % at 30 min for the generic products and the in silico simulation predicts similarity of the in vivo performance of these products. Computer simu-

▶ **Table 5** Inactive ingredients which were used in the brand and generic products.

Trade name	Inactive ingredients
Amlovasc®	Avesil pH 102, maize starch (sodium starch glycollate), dicalcium hydrogen phosphate, magnesium stearate.
Norvasc®	Microcrystalline cellulose, calcium hydrogen phosphate (anhydrous), sodium starch glycollate, magnesium stearate.

▶ **Table 6** In silico predicted and in vivo observed pharmacokinetics of amlodipine.

Parameter	Norvasc			Amlovasc
	Observed	Simulated	%PE	Simulated
C_{max} (ng/ml)	3.2	3.034	5.18	3.043
AUC $_{0\text{-inf}}$ (ng h/ml)	160.56	152.68	4.9	169.88

lations used in this study also predict similarity in the in vivo performance of these products.

Conclusion

In this study, gastrointestinal simulation technology was used to provide a justification for a biowaiver for amlodipine (A BCS class I) from immediate-release drug products. Our study showed that Amlovasc (5 mg/tablet) exhibit a similarity factor higher than 50 and a dissimilarity factor lower than 15. Therefore, the In vitro dissolution data provide a support for the simulation results. In addition, the tested products contain the same excipient as the brand one. Accordingly, a decision to waive these generics from in vivo bioequivalence studies can be justified as per the ICH guidelines. The use of these criteria in post-marketing of AM generics can also be suggested.

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Conflict of Interest

The authors have no conflict of interest to declare.

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