Journal of Pharmaceutical and Biomedical Analysis 47 (2008) 934–937

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Stability and *in vitro* release profile of enalapril maleate from different commercially available tablets: Possible therapeutic implications

Dione Marçal Lima, Leandro Dias dos Santos, Eliana Martins Lima*

Laboratório de Tecnologia Farmacêutica e Sistemas de Liberação de Fármacos, Faculdade de Farmácia, Universidade Federal de Goiás, 74605-220 Goiânia, GO, Brazil

ARTICLE INFO

Article history:
Received 11 December 2007
Received in revised form 21 February 2008
Accepted 23 February 2008
Available online 4 March 2008

Keywords:
Stability
Enalapril
Pharmaceutical dosage forms
Drug release

ABSTRACT

Stability of enalapril maleate formulations can be affected when the product is exposed to higher temperature and humidity, with the formation of two main degradation products: enalaprilat and a diketopiperazine derivative. In this work, stability and drug release profiles of 20 mg enalapril maleate tablets (reference, generic and similar products) were evaluated. After 180 days of the accelerated stability testing, most products did not exhibit the specified amount of drug. Additionally, drug release profiles were markedly different from that of the reference product, mainly due to drug degradation. Changes in drug concentration and drug release profile of enalapril formulations are strong indicators of a compromised bioavailability, with possible interferences on the therapeutic response for this drug.

1. Introduction

Stability of a pharmaceutical formulation can be considered a major factor in ensuring the quality of the drug product and consequently, the efficacy of the treatment [1,2]. Several parameters can influence the stability of a pharmaceutical product. These include the exposure of the product to a number of environmental conditions such as temperature, humidity and light, chemical composition, physical–chemical properties and quantity of formulation ingredients (both drug and excipients) and the manufacturing process including storage and conditions during transportation [3,4].

The drug release rate is one of the most important parameters for solid oral drug delivery systems, therefore, the therapeutic response is a function of the concentration of the drug available to be absorbed and reach the blood stream. Changes in the drug release profile will affect the absorption rate and thus, affect the therapeutic efficacy [1,5].

Worldwide adopted regulations [6–8] define that generic and reference drug products must be proven equivalent in both *in vitro* and *in vivo* studies and, as a result, can be interchangeable. When developing a generic drug formulation, the manufacturer must ensure that all *in vitro* specification requirements for the reference product are met. However, formulation excipients and manufacturing process may differ, as long as the bioequivalence between generic and reference products is not affected [5,9]. When requesting approval for similar products, manufacturers must provide technical data on stability and a bioavailability report. However bioequivalence between similar and reference products is not a requirement [10]. Generic drug policies have been established to broaden choices for pharmaceutical products with identical therapeutic profiles at a lower cost. However, it is imperative that generic substitutions do not compromise the safety and efficacy of the treatment. This remains a major controversy [11,12].

Enalapril maleate (Fig. 1) is a pro-drug without direct biological activity which is rapidly absorbed after oral administration and de-esterified *in vivo* to its active metabolite enalaprilat, a potent ACE (angiotensin-converting enzyme) inhibitor [13,14] widely used in the treatment of essential and renovascular hypertension and congestive heart failure [15–17].

Several studies indicate that enalapril stability can be altered when the drug is exposed to higher levels of temperature and humidity, leading to the formation of two major degradation products: enalaprilat (by hydrolysis) and a diketopiperazine (DKP) degradation product (by intramolecular cyclization) (Fig. 1) [14,18–20].

Enalapril tablets are available in the Brazilian market in three different categories: reference, generic or similar products, based on their approval by the national regulatory agency ANVISA.[1]

* Corresponding author. Tel.: +55 62 3209 6039; fax: +55 62 3209 6037.
E-mail address: emlima@farmacia.ufg.br (E.M. Lima).

[1] ANVISA – Agência Nacional de Vigilância Sanitária, Brazil (www.anvisa.gov.br).

0731-7085/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2008.02.030

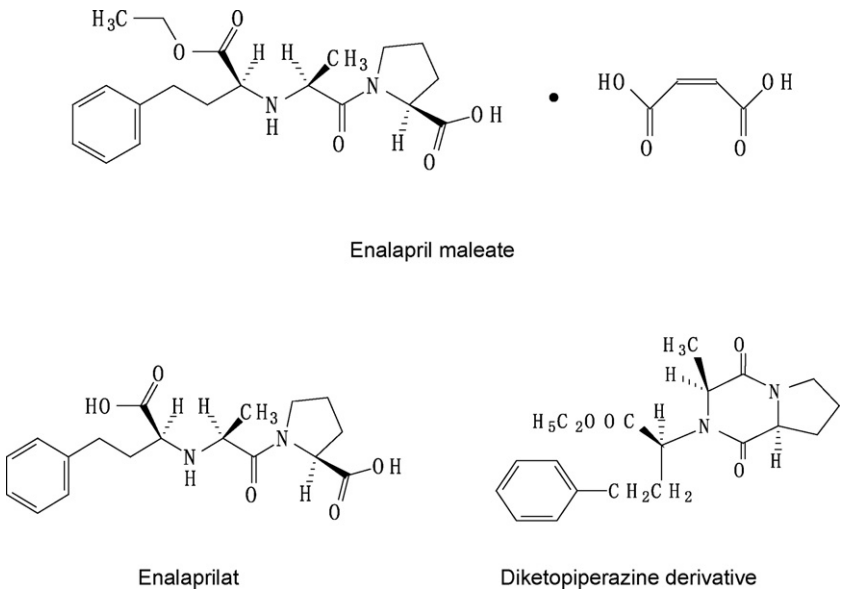

Fig. 1. Chemical structures of enalapril maleate, enalaprilat and diketopiperazine derivative.

In this work, the stability of different formulations of enalapril tablets was evaluated according to the ICH stability testing guidance [21]. Drug content and *in vitro* dissolution profiles were assessed at each referenced interval.

2. Experimental

2.1. Materials

Enalapril maleate and enalaprilat reference standards were purchased from the United States Pharmacopeia (Rockville, MD, USA). Enalapril tablets (20 mg of enalapril maleate) were purchased from local market pharmacies and were labeled as R (reference product); GA, GB, GC and GD (generic products); SA, SB, SE and SF (similar products). GA, SA and GB, SB were from the same manufacturer, respectively. ACS grade monobasic sodium phosphate, potassium phosphate and sodium hydroxide were purchased from Vetec (Duque de Caxias, RJ, Brazil). Phosphoric acid 85% was from Synth (Diadema, SP, Brazil). HPLC grade acetonitrile was purchased from JT Baker (Philipsburg, NJ, USA). HPLC ready 18 mΩ water was obtained from a Milli-Q Gradient A-10 water purification system, Millipore Corp. (Bedford, MA, USA).

2.2. Stability test protocol

Accelerated stability studies were conducted according to ICH stability testing guidelines [20] and ANVISA Resolution 1/2005 [22]. Tablets in their original packaging (aluminum blister) were stored in a stability testing chamber Nova Etica, 420 CLD (Sao Paulo, SP, Brazil), under controlled temperature ($40 \pm 2\,^{\circ}C$) and relative humidity ($75 \pm 5\%$). Samples were withdrawn at 0, 30, 90 and 180 days of the test and assayed for their drug content and *in vitro* drug release profile.

2.3. Instrumentation and chromatographic conditions

The HPLC system consisted of a Varian ProStar 240 series (Varian Inc., Palo Alto, CA, USA), equipped with a quaternary pump, column heater, auto-sampler and UV detector. Data collection and analysis were performed using Star Workstation software (Varian Inc., Palo Alto, CA, USA). Separation was achieved on a C18 Chrompack Reversed-Phase column 250 mm × 4.6 mm, 5 μm (Varian Inc., Palo Alto, CA, USA). Mobile phase was acetonitrile/10 mM NaH_2PO_4 (pH 2.2) (25:75, v/v), isocratic elution with a flow of 1.5 mL/min. The column temperature was maintained at 60 °C. Injection volume was 10 μL and UV detection at 215 nm.

2.4. Preparation of standard solutions

Enalapril maleate stock solution of 1 mg/mL was prepared in a 10 mM sodium phosphate buffer (pH 2.2) using the USP enalapril reference standard. Calibration standard solutions at five levels were prepared by serially diluting the stock solution for the analytical range of 0.02–0.3 mg/mL. Enalaprilat solution of 0.4 mg/mL was prepared in a 10 mM sodium phosphate buffer (pH 2.2) using the USP enalaprilat reference standard. DKP degradation product was produced from enalapril maleate reference standard, according to the USP method [23].

2.5. Sample preparation of the marketed tablets

Ten tablets of each commercial product were individually weighed, combined and ground into a fine powder using a glass mortar and pestle. A portion equivalent to the average weight of the 10 tablets was accurately weighed and transferred to a 100 mL volumetric flask. Volume was adjusted with 10 mM sodium phosphate buffer (pH 2.2). Samples were filtered through a 0.45 μm PVDF membrane filter, Millipore Corp. (Bedford, MA, USA) before HPLC analysis.

2.6. In vitro drug release assay (dissolution test)

The dissolution test was performed with a VK7000 Total Solution System, Varian Inc. (Cary, NC, USA) equipped with rotary paddles (USP apparatus 2) maintained at 50 rpm, auto-sampler and flow-through cell UV detection. Data collection and analysis were performed using CaryWin software, Varian Inc. (Cary, NC, USA). Dissolution media consisted of 900 mL of 50 mM KH_2PO_4 buffer (pH 6.8), at $37 \pm 0.5\,^{\circ}C$. Samples were assayed at 0, 5, 10, 15, 20, 25 and 30 min of the test.

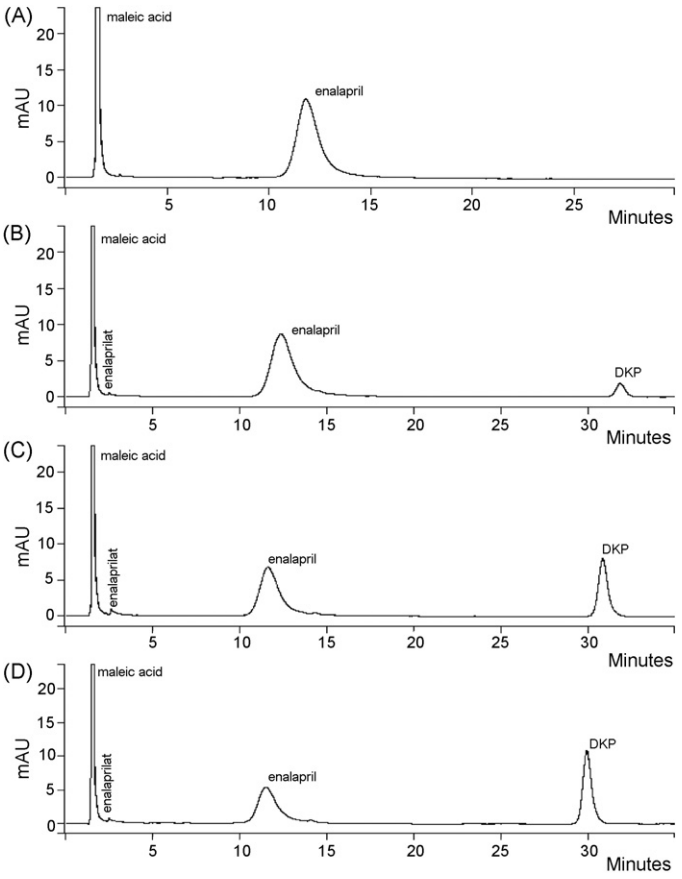

Fig. 2. HPLC chromatograms from enalapril (RT 11.5 min) and its degradation products enalaprilat (RT 2.5 min) and diketopiperazine derivative (DKP) (RT 31 min), obtained from sample GA at $t = 0$ (A), $t = 30$ (B), $t = 90$ (C) and $t = 180$ days (D) of stability test. C18 column at 60 °C, mobile phase: acetonitrile:sodium phosphate buffer 10 mM, pH 2.2 (25:75, v/v), 1.5 mL/min flow, UV detection at 215 nm.

Table 1
Quantitative determination of enalapril for each commercially available formulation during stability test after 0, 30, 90 and 180 days at a temperature of 40 ± 2 °C and a relative humidity of 75 ± 5%

Samples	Enalapril (mg)			
	$t = 0$	$t = 30$	$t = 90$	$t = 180$
Reference	20.30	19.84	19.65	18.83
GA	17.03	15.43	12.24	9.65
GB	20.38	19.84	19.76	18.18
GC	20.25	20.14	18.25	18.05
GD	19.41	17.91	15.20	12.97
SA	16.94	15.52	11.74	8.39
SB	20.26	19.40	19.40	18.54
SE	18.73	17.20	15.99	15.03
SF	20.08	19.56	16.80	13.58

t: time in days.

3. Results and discussion

The results obtained in this work confirmed that even commercially available formulations of this drug are highly susceptible to degradation. Fig. 2 exhibits chromatograms obtained from sample GA during accelerated stability studies, showing the gradual increase of enalapril degradation as a function of the time of exposure to higher temperature and humidity in the stability chamber. The main degradation product observed was the DKP derivative, indicating that humidity had no effect on the mechanism of the main degradation reaction [14]. Conversely, a higher proportion of enalaprilat was found by Al-Omari et al. [18] when relative humidity was >90%, but blistering of the tablets was able to reduce degradation even in the presence of 75% RH. The very low concentrations of enalaprilat found in this study are in agreement with these observations.

According to the United States Pharmacopeia [23], variations in drug content must not exceed ±10% of the labeled concentration. Thus, for the 20 mg enalapril tablets used in this work, any concentration lower than 18 mg/tablet is unacceptable. Additionally, variations between reference and generic products drug content must be within a 5% range [24]. Results of enalapril quantitative assay for each sample during the stability test are summarized in

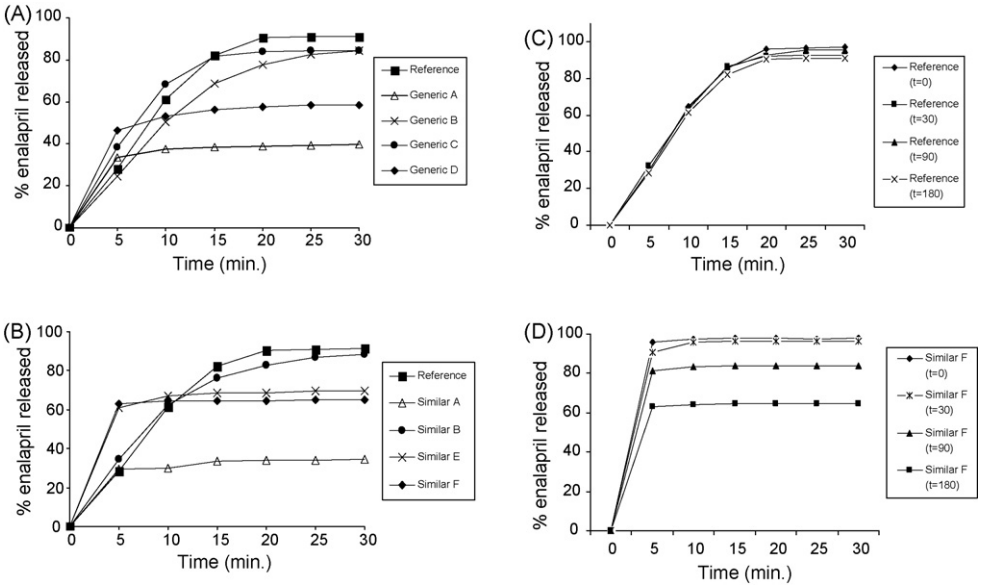

Fig. 3. Drug release profiles in 50 mM potassium phosphate buffer (pH 6.8): (A) reference and generic products and (B) reference and similar products after 180 days of the stability test; (C) reference and (D) similar products at each interval (in days) of the accelerated stability test in the stability chamber, temperature (40 ± 2 °C) and relative humidity (75 ± 5%).

Table 1. At time zero, i.e., before the initial phases of the test, two samples had drug contents out of specifications. Based on acceptance criteria of drug content within ±10% the labeled amount, unacceptable lower concentrations were found for samples GA and SA, both from the same manufacturer. At the end of the stability study (180 days), only four out of nine products tested were able to maintain enalapril dosage within the specified limits. *In vitro* release profiles obtained from all drug products after 180 days of the stability test are presented as percentage of drug released versus time in Fig. 3A and B. Again, only four products had a dissolution profile within the acceptable range ($Q \geq 80\%$ in 30 min) as established by the United States Pharmacopeia [23].

According to Dressman et al. [25], the dissolution test is considered the most accurate predictive method for the *in vivo* drug bioavailability of solid oral dosage forms. Marked differences in the kinetics of drug release can be observed in Fig. 3C and D. Reference product exhibited a gradual and continuous release throughout the dissolution test, with a release profile consistent with a zero-order kinetics. Drug release from sample SF was extremely fast, completed in less than 5 min of the dissolution test, not allowing further investigation of the release mechanism.

An initial burst release of a drug from its formulation may not be therapeutically desirable [25]. In these cases, it is necessary to evaluate if the organism is capable of absorbing the entire amount of drug released in the gastrointestinal fluid in a given period of time. This indicates that the differences in drug release profile observed throughout this study may result in changes in the bioavailability of enalapril from different formulations. Differences in the drug release profiles for these samples are probably a combined effect of different formulation ingredients and manufacturing processes [26,27].

4. Conclusions

Stability of enalapril maleate in tablet formulations may be adversely affected by temperature and humidity. Drug manufacturers and regulatory authorities must closely observe the recommendations made by the World Health Organization regarding drug stability tests for pharmaceutical products marketed in tropical or warm climate zones [28]. As shown in this study, drugs that are unstable under mild to moderate environmental conditions may result in a lower extent of bioavailability from their marketed formulations, possibly compromising therapeutic results.

References

[1] L.V. Allen, N.G. Popovich, H.C. Ansel, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, 8th ed., Lippincott Williams & Wilkins, 2004.
[2] P.G. Risha, C. Vervaet, G. Vergote, L. Van Bortel, J.P. Remon, Eur. J. Clin. Pharmacol. 59 (2003) 135–141.
[3] M. Lusina, T. Cindric, J. Tomaic, M. Peko, L. Pozaic, N. Musulin, Int. J. Pharm. 291 (2005) 127–137.
[4] K.S. Murthy, I.J. Ghebre-Sellassie, J. Pharm. Sci. 82 (1993) 113–126.
[5] S. Storpirtis, R. Marcolongo, F.S. Gasparotto, C.M. Vilanova, Infarma 16 (2004) 51–56.
[6] Public Law 9787: establishes generic drugs policy and regulates generic denominations for pharmaceutical products, Brazil, 1999.
[7] Guideline on registration requirements to establish interchangeability, WHO Technical Support Series, World Health Organization, 1996.
[8] Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products—general considerations, US Center for Rug Evaluation and Research, Food and Drug Administration, 2002.
[9] S.V. Dighe, Transplant. Proc. 31 (1999) 23S–24S.
[10] Guideline on registration requirements for similar products (Res. N. 133), ANVISA-MS, Brazil, 2003.
[11] P. Meredith, Clin. Ther. 25 (2003) 2875–2890.
[12] P. Crawford, M. Feely, A. Guberman, G. Kramer, Seizure 15 (2006) 165–176.
[13] R.J. MacFadyen, P.A. Meredith, H.L. Elliott, Clin. Pharmacokinet. 25 (1993) 274–282.
[14] B.J. Stanisz, J. Pharm. Biomed. Anal. 31 (2003) 375–380.
[15] T. Edeki, A. Johnston, E. Li Kam Wa, P. Turner, Int. J. Clin. Pharmacol. Ther. 32 (1994) 142–146.
[16] C.H. Espinel, J.L. Williams, S.S. Coughlin, Clin. Ther. 12 (1990) 181–190.
[17] T. Sato, M. Takeshi, A. Yamamoto, Circ. J. 56 (1992) 35–45.
[18] M.M. Al-Omari, M.K. Abdelah, A.A. Badwan, A.M. Jaber, J. Pharm. Biomed. Anal. 25 (2001) 893–902.
[19] X.Z. Qin, J. DeMarco, D.P. Ip, J. Chromatogr. A 707 (1995) 245–254.
[20] S.P. Bhardwaj, S. Singh, J. Pharm. Biomed. Anal. 46 (2008) 113–120.
[21] International Conference on Harmonization, ICH Q1 A (R2): stability testing of news drugs substance and products, published in Federal Register, 2003, pp. 16–31.
[22] Guideline on pharmaceutical stability studies (Res. N. 1), ANVISA-MS, Brazil, 2005.
[23] United States Pharmacopeia 27, National formulary, United States Pharmacopeia Convention, Rockville, 2004.
[24] Guideline on *in vitro* pharmaceutical equivalency and dissolution studies protocol (Res. N. 310), ANVISA-MS, Brasil, 2004.
[25] J.B. Dressman, G.L. Amidon, C. Reppas, V.P. Shah, Pharm. Res. 15 (1998) 11–22.
[26] K. Jackson, D. Young, S. Pant, Pharm. Sci. Technol. Today 3 (2000) 336–345.
[27] M. Albonico, P. Mathema, A. Montresor, B. Khakurel, V. Reggi, S. Pandey, L. Savioli, Trans. R. Soc. Trop. Med. Hyg. 101 (2007) 454–460.
[28] Recommendations on stability studies in a global environment, Document QAS/05.146, World Health Organization, 2004.