Original Paper

In Vitro and *In Vivo* Evaluation of Different Solid Dosage Forms Containing Captopril

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ABSTRACT: Aim. Comparison of Captopril generic formulations on the Romanian market with the reference formulation Capoten (Bristol Myers Squibb), in terms of *in vitro* release kinetics of active substance and *in vivo* pharmacokinetics. Materials and methods. Dissolution studies were performed using Apparatus 1 (Basket), DT 800H, Erweka, Germany in acidic medium (0.01 N hydrochloric acid) and an agitation speed of 50 rpm. Experiments were run on 12 tablets of each formulation. Quantification of Captopril was achieved by using a spectrophotometric method, λ=205nm. Clinical pharmacokinetics was determined in the frame of four different bioequivalence studies comparing a single dose four different Captopril 50mg generic tablet products to the innovator drug, Capoten 50mg (Bristol Myers Squibb). Results. Different batches of the reference formulations achieved dissolution profiles of the same form and very closed to each other at all dissolution points. Dissolution profiles of the tested formulations shown similar behavior for all references. Two generic formulations achieved a slower release at early dissolution time points, their release being "diffusion controlled", described by law of Higuchi. *In vivo*, products proved to be bioequivalent, but variability of space distribution and forms of plasma profiles was much bigger than for the in vitro release curves. Due to very rapid *in vitro* dissolution, a direct Level A *in vitro-in vivo* correlation was not possible, but, strangely, the fraction absorbed vs. time clearly followed the same Higuchi law. Conclusion. All the studied formulations achieved more than 85% dissolution after 15 minutes which means that whatever the values of dissolution metrics f1 and f2, formulations behave like a solution and generally should not have therapeutic equivalence problems. Slower dissolution profiles correlates with *in vivo* absorption being described by the same square root law of Higuchi which describe diffusion controlled transport phenomena.

KEYWORDS: *captopril formulations, release kinetics, bioequivalence, square root law*

Introduction

Captopril is an oral drug used for treating high blood pressure, heart failure, and for preventing kidney failure due to high blood pressure and diabetes. It has high water solubility and is readily absorbed after oral administration, 60-75% of the dose being absorbed and peak plasma concentrations are achieved within 1h [1].

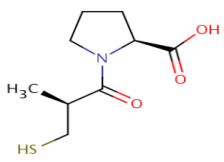

Fig. 1. Chemical structure of Captopril

Chemically, Captopril is-(2S)-1-[(2S)-2-Methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid (Fig. 1). Following Biopharmaceutical Classification System (BCS), Captopril is reported as a class I (high solubility and high permeability) drug [2]. However, other authors considered that it falls into class III [3,4]. In first case, dissolution is the rate limiting step of bioavailability and if dissolution is rapid then gastric emptying rate becomes the rate determining step.

In the case of class III drugs (low permeability, high solubility), the extent of absorption is limited by the permeation rate across intestinal epithelium. These drugs exhibit a high variation in the rate and extent of absorption.

In spite of scientific evidences, Romania is theater of a huge campaign against generic drugs. In the United States of America (USA), 90% of prescriptions refer to generic drugs. In Romania the situation is completely different, more than 80% of the drug market belonging to innovator drugs [5] so that proving the quality of generic drugs is a national priority.

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This study concerns the quality of Captopril formulations (Captopril 50mg, tablets) available in Romania by means of *in vitro* disposition and evaluates correlation to the *in vivo* data.

Materials and Methods

Captopril reference standard was purchased from Sigma-Aldrich. Captopril 50mg tablets, different generics, as well as the reference Capoten 50mg (Bristol Myers Squibb) tablets were purchased from a community pharmacy.

The following method and tolerances were applied (based on United States Pharmacopoeia (USP), under Captopril Tablets Monograph) [6].

Dissolution

Dissolution studies were performed in the frame of four different bioequivalence studies. Drug release experiments were performed using USP Apparatus 1 (Basket), DT 800H, Erweka, Germany. Each vessel was filled with 900mL of dissolution medium (0.01 N hydrochloric acid) and an agitation speed of 50rpm was used in all cases. Experiments were run on 12 tablets of each formulation. Samples (5mL) were removed after 5, 10, 15, 20min using a glass syringe, and then filtered through a 0.45-μm Teflon® filter. Quantification of Captopril was achieved by using a spectrophotometric method, λ=205nm.

Clinical study

The paper is in fact a meta-analysis based on four different bioequivalence studies (BE) which compared four Captopril formulation produced by Romanian companies (generics) with the brand drug, Capoten 50mg (Bristol Myers Squibb). All the four studies were conducted in the Emergency Clinical Military Hospital, Bucharest, Romania. The studies conformed to the Helsinki Declaration of 1964, as revised in 2013, and the entire study documentation was approved by the Ethical Committee of Biopharmacy & Pharmacol Res. S.A. and Romanian National Agency for Medicines and Medical Devices (ANMDM). The studies were conducted according to International Conference on Harmonization (ICH) Good Clinical Practices. All participants gave written informed consent prior to study participation. The final reports concerning studies together with the approval of Ethical Committee were validated by ANMDM and all the four generic Captopril formulations received market authorizations.

24 healthy male and female patients, aged between 18 and 55 years and with a Body Mass Index (BMI) between 19 and 25, with no prior history of alcohol and drug abuse were enrolled, and at least 20 of them completed each study. The subjects were excluded if they had a history of hypersensitivity to Captopril or to the drugs within the same pharmacological/chemical class, any clinically significant history of ongoing gastro-intestinal problems or problems known to interfere with ADME of drugs, history of clinically significant cardiovascular, renal, hepatic, pulmonary, metabolic, endocrine, hematological, gastrointestinal, neurological, psychiatric or other major diseases, HIV or hepatitis B or C positive testing, gastro-intestinal accelerated transit known to interfere with the absorption of the studied drug, if they had any clinically significant illness within the last 4 weeks, if they were under any prescribed systemic or topical medication within the last 2 weeks, if they took any drug within the last 1 week, or if they were part of any clinical trial within the last 2 months (independently from that approved or new investigational drug was tested).

Each study was designed as a single-dose, randomized, two-treatments, two-periods, two-sequence crossover study under fasting conditions with a washout interval of at least six days. Venous blood samples (5mL) were collected through a catheter inserted in the antecubital vein before (time 0) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6 and 8 hours after drug administration. Blood samples were centrifuged at 5°C for 6 minutes at approx. 3000rpm. Plasma was separated in two equal aliquots (1.2-1.3mL), immediately derivatized with monobromobimane in order to prevent Captopril dimerization [7], transferred to labelled 1.5mL polypropylene tubes and immediately frozen and stored at a <-20°C until analysis. Plasma levels of active substance Captopril were determined using a validated chromatographic method [7].

Results and Discussion

In Vitro Dissolution

Dissolution profiles obtained in the four bioequivalence experiments-Captopril 50mg generic formulations (TI, TII, TIII, TIV) versus brand formulation (different batches) Capoten 50mg (RI, RII, RIII, RIV) are presented in Fig. 2 and Fig. 3.

First comparison concerned the curves corresponding to four different batches of reference formulations (Fig. 2).

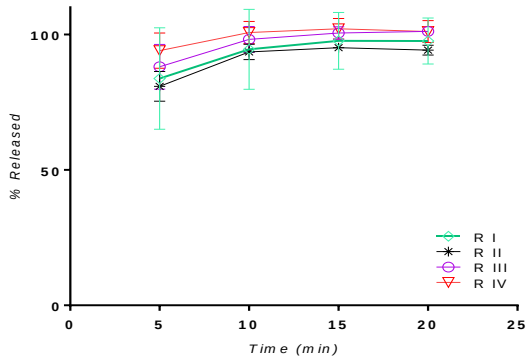

Fig. 2. Comparative dissolution profiles of four different reference (Capoten 50mg, Bristol Myers Squibb) batches (Mean±Standard Deviation; n=12 tablets)

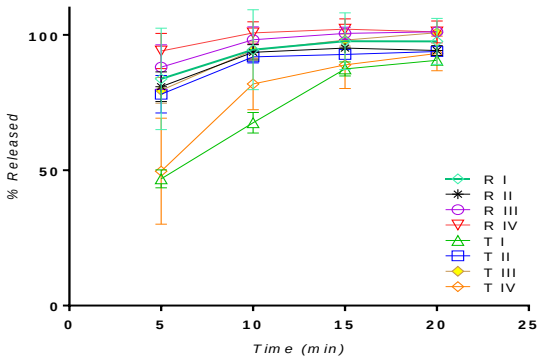

Fig. 3. Comparative dissolution profiles for all the analyzed Captopril tablets (Mean±Standard Deviation; n=12 tablets)

Different batches of the reference formulation achieved dissolution profiles having the same form and were closed at every dissolution point to each other, proving a low variability between products. Conclusion is confirmed by descriptive statistics, variation coefficient being found between 2.9 and 5.7%.

Reference drug-Tested drugs comparisons. Application of similarity and dissimilarity metrics for intercurves comparisons

The release profiles were tested concerning similarity or non-similarity using f_1 (difference) and f_2 (similarity) metrics:

$$f_1 = \frac{\sum(R_i - T_i)}{\sum T_i} \quad \text{and} \quad f_2 = 50\log\left\{\left[1+\frac{\sum_{i=1}^{p}\left(\overline{x_{ti}} - \overline{x_{ri}}\right)^2}{P}\right]^{-1/2} * 100\right\}$$

Where R_i and T_i represent the experimental percent dissolution values obtained for the reference and tested drug; P-number of measured data.

The threshold between similarity and non-similarity is considered as 10%. If the difference is 10% in all points, a f_2 value 50 is obtained. For this reason, if the f_2 value is greater than 50, the curves are considered similar. If the obtained value is lower than 50 it is accepted the hypothesis of dissimilarity. f_1 has not a threshold. If dissolution profiles are identical, f_1 =0. If dissolution of reference drug is very slow, f_1 tends to infinity.

All formulations met the USP 32 dissolution specifications which indicate that not less than 80% of the labeled amount of Captopril dissolved in 20 minutes (Fig. 4).

Dissolution profiles of the studied formulations shown similar behavior for all the reference batches, with a minimum f_2 value of 69,19. In order to perform pairwise comparisons between all the analyzed product, a mean profile for the four batches of reference formulation was used.

Dissolution profiles of the studied formulations shown similar behavior for Reference, T II and TIII formulations, whilst TI and TIV formulations achieved a slower release at the early time dissolution points (Table 1).

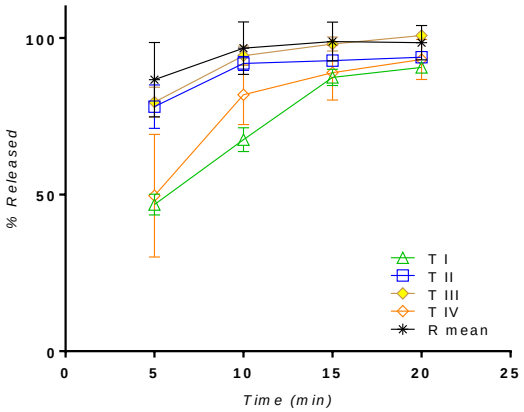

Fig. 4. Mean dissolution curves of reference (Mean of four different batches) and four generic formulations

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Table 1. Pairwise comparison by means of f2 similarity factor between the analyzed Captopril products

Formulation	R_{mean}	T I	T II	T III	T IV
R	100	29.52	59.93	69.43	34.16
T I	29.52	100	34.87	32.49	56.20
T II	59.93	34.87	100	66.43	40.90
T III	69.43	32.49	66.43	100	38.15
T IV	34.16	56.20	40.90	38.15	100

The BCS suggests that for high solubility, high permeability (class 1) drugs and in some instances for high solubility, low permeability (class 3) drugs, more than 85% dissolution in 15 minutes can ensure that the bioavailability of the drug is not limited by dissolution. In these cases, the rate limiting step for drug absorption is gastric emptying.

All the evaluated formulations achieved more than 85% dissolution at 15 minutes. The differences, though mathematically and statistically significant, does not imply differences in terms of *in vivo* release and pharmacokinetics. Captopril different formulations have a behavior closed to oral solutions and are as a rule, similar in terms of release [10,11].

In case of the formulations with slower release (TI and TIV), application of release kinetics models in order to estimate the mechanism of dissolution and release was attempted.

First tested model was Higuchi's law, which in fact is a more general, square root law [8]:

$$m = \sqrt{DS(2M - S)t} = \alpha t^{1/2}$$

where D is the diffusion coefficient, S the solubility of active substance and M the total mass of active substance included in tablet. The model is considered valid if a linear dependence of the drug released quantity as function of square root of time $R(\sqrt{t})$ is obtained. For both T I and T IV such linear dependence was obtained (Fig. 5a).

Higuchi model is based essentially on diffusion mechanism. Another model, which assumes swelling of the tablet matrix in parallel with diffusion, was proposed by Peppas [9]:

$$M(t) = at^n$$

where a and n are constants. It is to underline that n is not necessary an integer number.

If Peppas model is applicable, representation of natural logarithm of released quantity as function of *ln t* can be well approximated by a straight line. Such a dependence was also obtained (Fig. 5b), but the correlation is poor than in case of square root time model.

In conclusion release kinetics seems to follow a "diffusion controlled" mechanism. Really, the representation of released amount as function of square root of time proved a very good linear regression. Additionally, the linear regression starts clearly from origin, i.e. the lag-time is practically zero: release starts instantaneously. Application of Peppas model is less clear and Higuchi model is simpler, and consequently preferable.

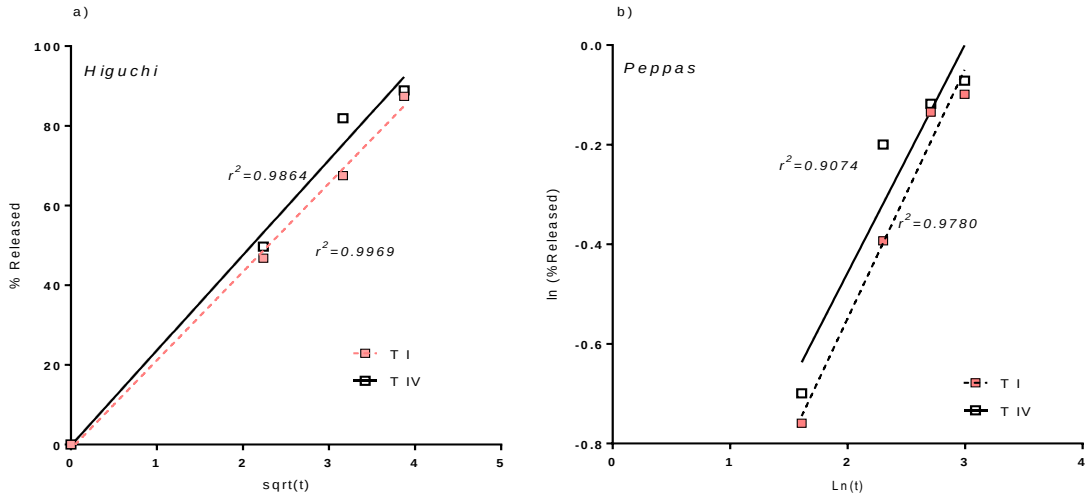

Fig. 5. Modelling of in vitro release kinetics for TI and TIV products. a. square root model, b, Peppas model

In vivo dissolution

As a rule, small differences in terms of in vitro dissolution, does not necessarily implies differences of the *in vivo* dissolution.

The four different bioequivalence studies taken into account in the present paper concluded that all the generic formulations were bioequivalent to reference. Although *in vivo* products proved to be bioequivalent, as expected variability of space distribution and forms of plasma profiles was higher than *in vitro* release curves. Taking as model the individual curves of Capoten 50mg obtained in one of the four studies, it can be seen that the profiles are grouped in a more or less homogeneous cluster with exception of three curves, outliers with respect to maximum concentration (Subject 12) or time of the maximum concentration (Subjects 2 and 7). If these outliers are connected with release or to absorption, is difficult to say, but it is clear that, following its polar nature, Captopril is not so easily absorbed.

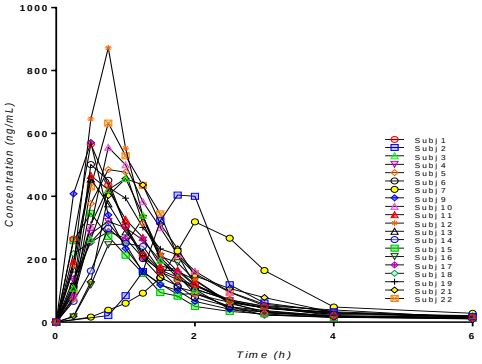

Fig. 6. Individual pharmacokinetic profiles obtained after administration of a single dose of Capoten 50 mg (Bristol Myers Squibb) to 20 healthy volunteers

The mean values for the main pharmacokinetic parameters are presented in Table 2.

Table 2. Mean pharmacokinetic parameters obtained after administration of Capoten 50mg to 20 healthy volunteers

Parameter	UM	Mean	SD	%CV
Cmax	**ng/mL**	453.35	142.22	31.37
Tmax	**h**	0.84	0.41	48.69
AUCtot	**ng/mL*h**	625.66	134.78	21.54
Ke	**1/h**	658.04	144.77	22.00
AUMCtot	**ng/mL*(h)²**	0.54	0.26	47.78
thalf	**h**	1320.60	538.52	40.78
MRT	**h**	1.76	1.26	71.17
Clearance/F	**L/h**	1.98	0.61	30.63
Vz/F	**L**	79.56	17.63	22.16

Due to very rapid *in vitro* dissolution, a Level A it *in vitro-in vivo* correlation but was not possible. However, Wagner-Nelson [12] formula for calculation of absorbed fraction of Captopril as function of time was applied:

$$FRA(t_i) = \frac{c(t_i) + \int_0^{t_i} k_e c dt}{\int_0^{\infty} k_e c dt} = \frac{c(t_i) + k_e AUC_{0-t_i}}{k_e AUC_{0-\infty}}$$

where, $FRA(t_i)$ is fraction of the drug absorbed at time t_i; $c(t_i)$ -plasma concentration of drug at time t_i; k_e -elimination rate constant; AUC_{0-t_i} -area under the concentration-time curve time 0 to time t_i; $AUC_{0-\infty}$ -area under the concentration-time curve of the drug from time 0 to infinity.

In some particular initial and boundary conditions mathematical equation of diffusion could be solved and quantity transferred across interfaces [13] is linearly dependent on square root of time, law similar to that of Higuchi concerning release from solid dosage forms, Using a mathematical model which beyond the frame of present paper it was predicted the possibility that absorbed fraction calculated using Wagner -Nelson formula to be proportional to square root of time.

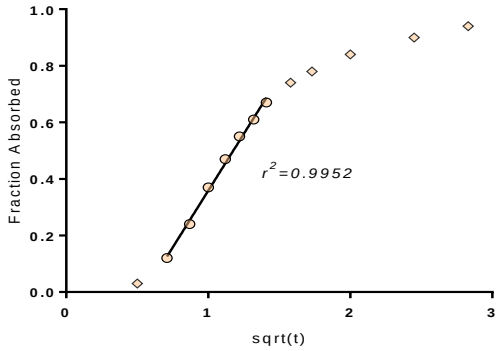

Fig. 7. Linear dependence of FRA of square root of time

Calculation of FRA from mean curve of clinical experiment confirmed the theoretical prediction (Fig. 7) for the time interval from administration to time of maximum concentration.

These results are of a great generality and will be discussed in detail in other, more theoretical papers.

Following these type of results, Food and Drug Administration (FDA) suggested in 2016, possibility of waiver request of *in vivo* testing for example for 12.5mg, 25mg and 50mg strengths based on (i) acceptable bioequivalence study on

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the 100mg strength, (ii) proportional similarity of the formulations across all strengths, and (iii) acceptable *in vitro* dissolution testing of all strengths [14].

On the contrary, these positive results do not imply automatically bioequivalence of all Captopril formulations. *In vitro* dissolution is not predicting *in vivo* dissolution and absoption. Weak bases for example are released very quickly in gastric fluid and precipitate at intestinal pH. This is the reason that, during the last years, dissolution tests are conducted in "biorelevant media" proved to be representative for the fasted stomach (FaSSGF), the postprandial stomach (FeSSGF), fasting state conditions in the small intestine (FaSSIF) and simulated postprandial conditions in the small intestine (FeSSIF) [15,16].

Conclusions

The release of Captopril from all formulations was in the required limits for immediate release products.

The reference formulations different batches achieved much closed dissolution profiles providing a low variability.

Generic formulations TI and TIV had a more slow release on the early dissolution time points, following a square root law model. Generic formulations TII and TIII have very closed release profiles maybe due to same production site or similar excipients and formulation equipments.

All the studied formulations achieved more than 85% dissolution within 15 minutes, which means that whatever the values of f_2, formulation behave like a solution and generally should not have any bioavailability problems.

The quality of the Captopril formulations available in Romania are in the required limits proved by the *in vitro* evaluation and also sustained by the pharmacokinetic data from the clinical studies.

The belief of many patients and even clinical doctors that there are therapeutic differences between brand and generic Captopril formulations is false. The results obtained exclude practically the possibility of differences in therapeutic effect.

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