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Original scientific paper

Biorelevant dissolution of candesartan cilexetil

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Abstract

The choice of an appropriate medium for dissolution tests is an essential step during a dosage form development. The adequate design of dissolution testing enables forecasting in vivo behavior of drug formulation. Biorelevant media were developed for this purpose because dissolution media described in the International Pharmacopoeia are not thoroughly suitable. Therefore, we carried out solubility and dissolution tests in biorelevant media and we compared the results with data measured in compendial dissolution media. A shake-flask method and standard paddle apparatus were used. The concentration was measured by a UV-Vis spectrophotometer. An oral solid dosage form with poorly soluble drug candesartan cilexetil was tested. Significant differences in the solubility and dissolution profiles of candesartan cilexetil were observed. The study offers the overview of compendial and biorelevant media simulating fasted state that can be analyzed by a spectrophotometric technique.

Keywords

dissolution test; compendial medium; biorelevant medium; fasted state; poorly soluble drug; candesartan cilexetil.

Introduction

During a drug dosage form development it is essential to investigate factors which influence drug absorption, especially after oral administration. The prediction of limiting factors can be facilitated by *in vitro* tests [1]. *In vitro* dissolution tests should mimic a drug performance in a human proximal gastrointestinal tract (GIT). To establish reliable *in vitro* testing it is important that artificial environments simulate physiological conditions as closely as possible. The level of the simulation is dependent on many factors related to used equipment and medium. A physiological relevant dissolution medium is great contribution to *in vitro* dissolution tests [2].

Dissolution media were initially intended mainly for quality control purposes and water was frequently used as the dissolution medium. However, later approach led to the development of new media which resemble gastrointestinal fluids. Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF) were the first proposals [3]. Both artificial fluids are described in the International Pharmacopoeias. These media reflect roughly pH conditions and the enzyme activity in the stomach (SGF contains pepsin, pH 1.2) and small intestinal (SIF contains pancreatin, pH 6.8). Nevertheless, there are variants without enzymes – SGF_{sp} (sine pepsin) and SIF_{sp} (sine pancreatin) [4]. SGF and SIF do not contain any surfactants, although

surfactants have been commonly used to improve the solubility of low soluble drugs. Therefore, Dressman et al. [5] and Galia et al. [6] proposed addition of synthetic surfactants – Triton X®-100 (SGF_{Triton}) and sodium lauryl sulfate (SGF_{SLS}). These media induced better solubility of low soluble compounds but on the other hand they were not physiologically relevant [7].

Although the composition of artificial fluids reflects specific parameters of GIT physiology, they are not adequate models of *in vivo* conditions [2]. Thus, Fasted State Simulated Gastric Fluid (FaSSGF) and Fasted State Simulated Intestinal Fluid (FaSSIF) have been regarded as biorelevant media. FaSSGF was proposed by Vertzoni et al. in 2005 [8] and FaSSIF by Galia at al. in 1998 [4]. Both biorelevant media contain natural surfactants: taurocholate sodium, which represents bile salts, and lecithin. The medium FaSSGF comprises the enzyme pepsin as the medium SGF but pepsin is contained in very low concentration in FaSSGF. Unlike SIF, FaSSIF does not comprise the enzyme pancreatin.

All reported media were developed for the simulation of fasting conditions in the upper GIT. Media simulating fed state of stomach (Fed State Simulated Gastric Fluid, FeSSGF) and small intestine (Fed State Simulated Intestine Fluid, FeSSIF) have been also developed. Former researches about the simulation of postprandial gastric conditions tested homogenized breakfasts with milk and nutrition products [2]. The first proposal of FeSSIF was published by Galia at al. in 1998 [4]. Updates media (snapshot media) were proposed by Jantratid et al. in 2008 [9]. However, this study is not interested in fed state media since their composition hinders direct spectrophotometric analysis.

The biorelevant dissolution properties of many drugs have been studied since the beginning of biorelevant media development. Studies of solubility in biorelevant media were carried out to predict bioavailability particularly of ionisable molecules since the solubility of ionisable compounds depends on their pKa and pH of the media [10]. The solubilizing effect of bile salts, lecithin and other food effects on drug solubilization were tested for instance by Takács-Novák et al. [10], Frank et al. [11], Dressman at al. [12], and Kostewicz et al. [13].

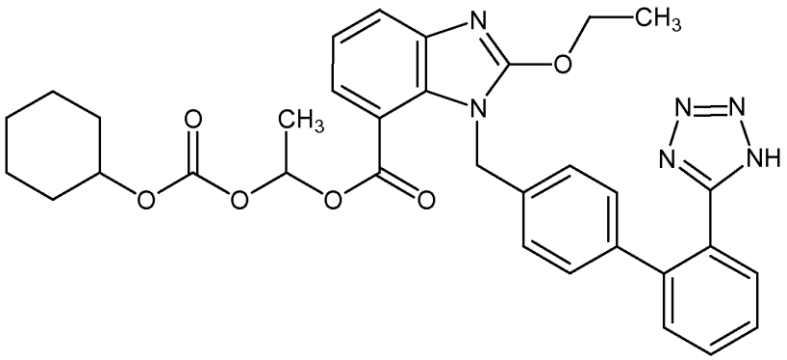

Figure 1. Structure of candesartan cilexetil (CC)

The precise imitation of the *in vivo* conditions is desirable particularly for poorly soluble drugs. As a case example of poorly soluble drug candesartan cilexetil (Figure 1) was chosen which is practically insoluble in water (less than 0.05 μg/ml [14]). As prodrug, candesartan cilexetil (CC) is completely bioactivated by ester hydrolysis to candesartan during a gastrointestinal absorption. Actually, candesartan is an angiotensin II receptor antagonist and it is mainly indicated for the treatment of hypertension [15]. However, CC is classified as BCS Class II drug and its very low solubility across the physiological pH range brings about an incomplete absorption and it is a reason for low bioavailability (about of 15 % [14]). The compound of our interest is a weak acid (p*K*a 6.0 [16]) with lower solubility in low pHs due to deprotonation of a tetrazole group at pHs higher than 6 [16]. The results from our study confirm an increase in CC solubility with

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increasing pH. Moreover, the solubility of CC is enhanced using surfactant Tween 20 and the surfactant is required to achieve sink conditions. The dissolution testing of CC for quality controls has been standardly carried out in 900 ml of 0.05 M phosphate buffer (pH 6.5) with 0.35 % or 0.7 0% (w/w) Tween 20 (shortcut PPT20). The amount of Tween 20 depends on dosing conditions [17].

A big challenge with CC is its low stability in solutions. The most important factors affecting the stability of CC in solution are pH and temperature. Hoppe and Sznitowska [18] found out that the degradation rate of CC was faster at elevated temperatures and low pHs. According to their results, the half-life of degradation is only 35.91 hours in 0.1 M HCl (pH 1.2) and 150.7 hours in 0.05 M phosphate buffer (pH 6.5), both at 37 °C and without surfactants [18].

Our aim was to study and compare the dissolution properties of CC in media simulating the fasted state of stomach and small intestine. This paper provides the solubility data and dissolution profiles of CC in various media (artificial media with or without surfactant and enzyme, biorelevant media) which will be helpful for prediction of the *in vivo* performance of CC after oral administration.

Experimental

Materials

Candesartan cilexetil and immediate release (IR) tablets Carzap (product of Zentiva k.s., Czech Republic) with 8 mg of CC were obtained as gift samples. All chemicals used in the study were of analytical grade. The composition of the prepared media is presented in Table 1 and Table 2.

Table 1. Composition of gastric media.

Component	FaSSGF	SGF	SGF_{sp}	SGF_{SLS}	SGF_{Triton}
Sodium taurocholate (mM)	0.08	-	-	-	-
Lecithin (mM)	0.02	-	-	-	-
Sodium lauryl sulfate (%, w/v)	-	-	-	0.25	-
Triton X-100 (%, w/v)	-	-	-	-	0.10
Pepsin (mg/ml)	0.10	3.20	-	-	-
Sodium chloride (mM)	34.20	34.20	34.20	34.20	34.20
pH (adjusted by hydrochloric acid)	1.6	1.2	1.2	1.2	1.2

Table 2. Composition of intestinal media.

Component	FaSSIF	SIF_{sp}	PPT20
Sodium taurocholate (mM)	3.00	-	-
Lecithin (mM)	0.75	-	-
Tween 20 (%, w/w)	-	-	0.35
Pancreatin (mg/ml)	-	-	-
Monobasic sodium phosphate (mM)	28.36	-	-
Monobasic potassium phosphate (mM)	-	49.97	50.00
Sodium hydroxide (mM)	8.70	45.00	-
Sodium chloride (mM)	105.85	-	-
pH (adjusted by sodium hydroxide)	6.5	6.8	6.5

Methods

Solubility studies

The solubility of CC was determined by the shake-flask method. CC was added in excess into specific solvent and shaken for 24 hours at 37 °C in a thermostated orbital platform shaker (Heidoplh, Unimax 1010 with Incubator 1000) to obtain equilibrium solubility [18, 19]. Due to the low stability of CC, a 'shortened shake flask method' was suggest, the shaken time of certain saturated solutions, namely with solvents SGF, FaSSGF and SGF_{Triton}, was only 2 hours or 12 hours. The concentration of CC was measured by a UV-Vis spectrophotometer (Schimadzu UV Mini 1240) at 256 nm and before the analysis supernatant had been filtered through a 35 micron porous filter from UHMW polyethylene. All tests were carried out in triplicate.

Concentration of dissolved CC was determined from calibration curve. The stock solution of CC was prepared in the suitable quantity of methanol. The aliquot of this solution was diluted with the specific solvent to get the final concentration of standard solutions (10-30 μg/ml). The method obeyed Lambert--Beer law (r^2>0.99).

Dissolution studies

Dissolution tests were performed in a paddle apparatus (USP II). The stirring rate was 50 rpm. The dissolution medium was filtered through a 35 micron porous filter from UHMW polyethylene, pumped through a 10 mm flow cell by a peristaltic pump (Ismatic, Reglo Digital MS-2/6) and analyzed by a UV-Vis spectrophotometer (Schimadzu UV Mini 1240), wavelenght was 256 nm. All dissolution tests were conducted in triplicate. The tablets of CC were placed in 500 ml of dissolution medium (37±0.5 °C). The volume was set as the smallest amount which enables the performance of the dissolution testing in USP II [2]. To simulate the fasted state, the volume of media in the range of 250-300 ml (stomach) or 300-500 ml (duodenum) is recommended [8], but the volume reduction was not feasible. Despite the volume reduction, sink conditions were maintained in medium PPT20 regarding on our solubility data of CC. These data verify findings reported by Hoppe and Sznitowska [18].

Results and Discussion

Solubility study

The equilibrium solubility data (Table 3) confirm that CC is a poorly soluble and stable compound. The values of CC solubility in SGF after 24 hours of shaking were so small that it was not possible to record them by the UV-Vis spectrophotometer. Zero solubility in SGF and very low solubility in FaSSGF (0.72±0.19 μg/ml) were caused by the rapid CC degradation at the acid environment. Due to the long measurement time, the large amount of CC degraded. Because of the CC degradation, the shaking time was shortened to two hours for media SGF, FaSSGF and SGF_{Triton}. From the obtained data, we concluded that the shorter shaking time reduced the amount of emerging degradation products (CC saturation solubility was 2.76 μg/ml in SGF and 0.90 μg/ml in FaSSGF). But the equilibrium may not be always reached in such short time, as evidenced by almost ten times lower value of CC solubility in SGF_{Triton} (0.69 μg/ml after 2 hours compared to 6.00 μg/ml after 24 hours test). CC gained the greatest solubility in FaSSGF after 12-hour period of shaking (1.59 μg/ml). For these data, it can be concluded that 12 hours is an optimal period for the shake-flask method considering the solubility and degradation of CC in FaSSGF.

Very low amounts of CC were dissolved in SGF_{sp} and SIF_{sp}, 1.35 and 0.65 μg/ml, respectively. In this case, the low solubility of CC was probably the main reason of such results. We observed that the powder of CC

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was almost dry after the tests because in the media without enzymes and surfactants the required wetting of the samples was not reached. Satturwar et al. published the values of CC solubility in SGF (0.6 µg/ml) and SIF (8.6 µg/ml) [20]. However, the preparations of these media and the conditions of solubility study were not described in the research paper. Besides, we suppose that the used media did not contain enzymes, which means SGF_{sp} and SIF_{sp}, due to other procedures mentioned in the paper. Our solubility data of CC measured by a shake-flask method in SGF_{sp} and SIF_{sp} do not correspond with data by Satturwar et al. [20]. Our CC solubility in SGF_{sp} is twice as high and CC solubility in SIF_{sp} 13 times as low than Satturwar's data.

Table 3. Saturation solubility of CC in used media.

Medium	Solubility of CC (µg/ml)	
	Shortened test	Standard test (24-hour)
PPT20	-	93.00±0.10
FaSSGF	0.90±0.07* 1.59±0.02**	0.72±0.19
SGF	2.76±0.46*	-
SGF_{sp}	-	1.35±0.10
SGF_{Triton}	0.69±0.07*	6.00±0.25
SGF_{SLS}	-	147.63±0.35
SIF_{sp}	-	0.65±0.01
FaSSIF	-	8.26±0.18

* 2-hour test
**12-hour test

Results demonstrate that only in the media SGF_{SLS} (147.63 µg/ml) and PPT20 (93.00 µg/ml) sink conditions for 8 mg tablets of CC can be achieved in volume 500 ml. Three liters of FaSSIF and four liters of SGF_{Triton} were required to achieve minimum sink conditions (approximately 30 % of the saturation concentration of CC) in dissolution studies. However, so large volumes are not physiologically relevant. Based on solubility studies, it is expected that in the biorelevant media for fasted state conditions are not sufficient to achieve complete dissolution of the minimum dose.

Dissolution study

The dissolution properties of CC are influenced by pH due to the ionization effect of CC at pHs above 6.0, and also by the presence of a surfactant. The similar amount of CC (12-13 %) was dissolved in SGF and FaSSGF media (Figure 2) at pH 1.2 and 1.6, respectively. It was predictable from the results of saturation solubility. Dissolution testing proved the same dissolution rate of CC. Very small divergence between profiles of SGF and FaSSGF is caused by the close values of pH and the low concentration level of the natural surfactants in FaSSGF. Despite the fast degradation of CC at acid pH, the decrease of the CC concentration was not observed during one hour. However, 5 % decrease of the CC concentration was observed throughout the dissolution tests in SGF_{sp} (Figure 3). Less considerable, but still noticeable, 0.5% reduction occurred also in case of the dissolution CC in SIF_{sp}. These courses of the dissolution profiles in SGF_{sp} and SIF_{sp} prove the extremely short-time stability of CC in aqueous environment without surfactants or enzymes. The reason why the medium SIF_{sp} instead of SIF was used is that pancreatin coloured the medium and SIF could not be analyzed by a spectrophotometric technique. The amounts of dissolved CC in 35 minutes in SGF (12 %) and SIF_{sp} (10 %) were close to each other until the degradation process in SIF_{sp} occured. Similar dissolution profiles were achieved in spite of the low pH value in SGF (SGF-pH 1.2 and SIF_{sp}-pH 6.8, the highest pH of the used media) on the one hand and the low equilibrium solubility of CC in SIF_{sp} on the other hand. Nevertheless, the ionization of CC molecules at pHs above 6.0 probably occurred

during the dissolution test. The ionization resulted in solubility increase and subsequently the degradation of dissolved compound.

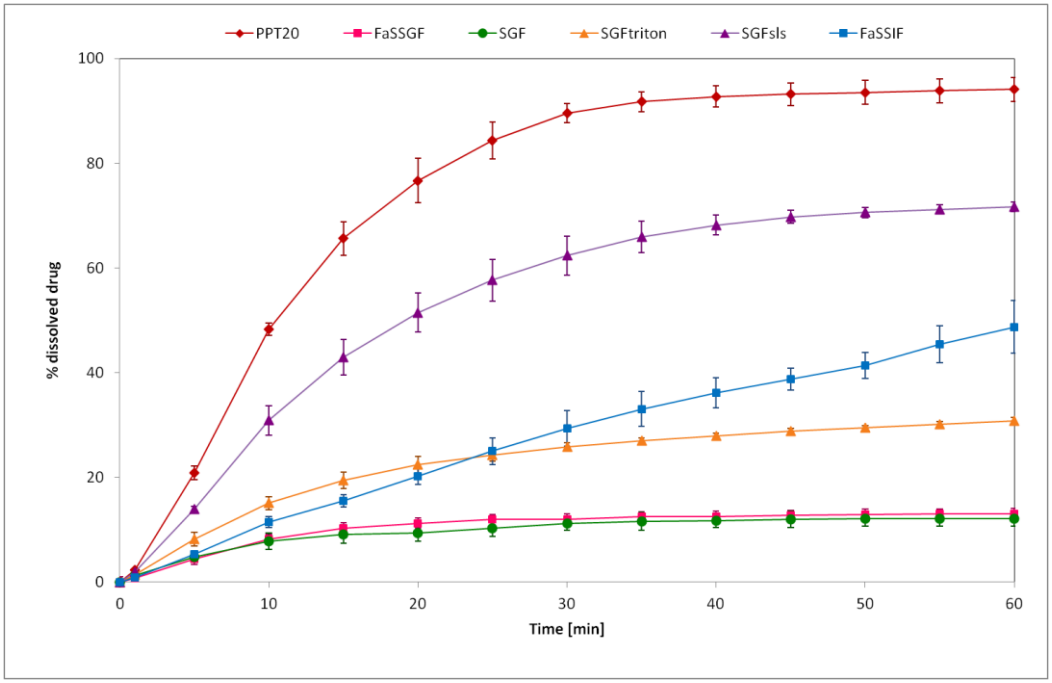

Figure 2. Dissolution profiles of candesartan cilexetil in medium PPT20, FaSSGF, SGF, SGF_{Triton}, SGF_{SLS} and FaSSIF

The most promising medium for the dissolution testing of CC was considered FaSSIF, the medium with pH 6.5 and the natural surfactants taurocholate sodium and lecithin. However, the CC solubility in this medium was not sufficient. For this reason, sink conditions were not obtained in the paddle apparatus and therefore, the dissolution rate and extent of the CC solubility (46 %) was reduced (Figure 2).

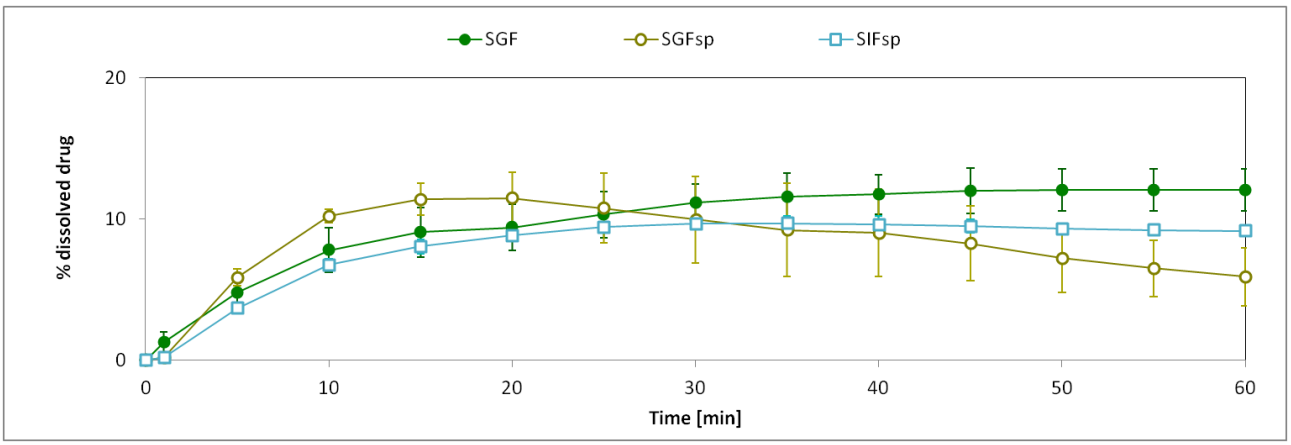

Figure 3. Dissolution profiles of candesartan cilexetil in medium SGF, SGF_{sp} and SIF_{sp}

The biorelevant dissolution profiles of CC are not accordant with the dissolution profiles of CC in the media with the artificial surfactants, especially with the surfactant Tween 20 and sodium lauryl sulfate (Figure 2). It was found for CC that solubilization plays much more determining role in solubility than ionization. The solubility and dissolution rate of CC in the media PPT20 (96 %) and SGF_{SLS} (72 %) are considerately higher. In the case of SGF_{SLS} it is despite the low pH value (pH 1.2). The increase of the solubility in the medium containing the surfactant Triton X®-100 is not so pronounced (31 %). This indicates

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how the choice of a surfactant influences the resulting solubility of a drug. It is evident that the application of dissolution media with artificial surfactants would lead to false positive results because artificial surfactants induce greater solubilization effects than what would be physiologically relevant.

Conclusions

The need for *in vitro* test which mimics *in vivo* conditions of the gastrointestinal tract led to the development of physiologically relevant dissolution media. The dissolution of the poorly soluble drug CC was studied in different type of dissolution media: the compendial medium (PPT20) commonly used for quality control, the artificial fluids with the synthetic surfactants (SGF_{Triton}, SGF_{SLS}) or without them (SGF, SGF_{sp}, SIF_{sp}) and the fasted state simulating biorelevant media (FaSSGF, FaSSIF). In this study, the dissolution media that are possible to analyse by a UV-Vis spectrophotometer were examined. Consequently, dissolution tests with medium SIF and biorelevant media simulated fed state (FeSSGF and FeSSIF) were not carried out.

The amount of dissolved CC in the biorelevant media was significantly lower than the amount of CC dissolved during tests with the media containing the synthetic surfactants. Whereas the biorelevant media contain natural surfactants as an alternative to the non-physiologically relevant surfactants, the dissolution profiles of CC corresponded with the *in vivo* behavior of CC closely in FaSSGF and FaSSIF. The poor solubility and stability of CC in the biorelevant media resulted in its low bioavailability. The results can be utilized as an example that the biorelevant media are useful to forecast the oral absorption of a BCS Class II drug.

List of abbreviations

CC	candesartan cilexetil
FaSSGF	fasted state simulated gastric fluid
FaSSIF	fasted state simulated intestinal fluid
FeSSGF	fed state simulated gastric fluid
FeSSIF	fed state simulated intestinal fluid
GIT	gastrointestinal tract
PPT20	phosphate buffer with surfactant Tween 20
SGF	simulated gastric fluid
SGF_{sp}	simulated gastric fluid sine pepsin
SGF_{SLS}	simulated gastric fluid without enzyme but with surfactant sodium lauryl sulfate
SGF_{Tritin}	simulated gastric fluid without enzyme but with surfactant TritonX® 100
SIF	simulated intestinal fluid
SIF_{sp}	simulated intestinal fluid sine pancreatin

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