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Development of Dissolution Medium for Candesartan Cilexetil by RP-HPLC Method

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ABSTRACT

The present study deals with the dissolution of an angiotensin II receptor antagonist drug, candesartan. Candesartan cilexetil is a poorly water-soluble prodrug. The *in vitro* dissolution testing of Candesartan cilexetil in water and buffer solutions is not possible. In the present study, an attempt was made to develop a dissolution medium for *in vitro* testing of the drug. A Kromacil C18, 5µm column having 150x4.6 mm internal diameter in isocratic mode with mobile phase containing mixture of buffer (pH 4.5) and acetonitrile in ratio of 45:55 was used. The flow rate was 1.5 mL/min and effluents were monitored by UV at 257 nm. The selection of the medium was made on the basis of solubility data of Candesartan cilexetil in different dissolution medium at 37 °C. Solubility data revealed that phosphate buffer (pH 6.5) consisting of 0.35% w/v tween 20 could be a suitable dissolution medium.

Key words: Candesartan, solubility, buffer, dissolution.

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INTRODUCTION

Candesartan cilexetil belongs to the class of angiotensin receptor antagonist and acts by binding selectively and non-competitively to angiotensin II receptor type 1, thus preventing actions of angiotensin II. The drug finds most significant clinical uses in the treatment of hypertension of all grades. Candesartan cilexetil is an ester prodrug of its active metabolite candesartan, to which it owes its therapeutic effect[1]. Candesartan cilexetil is chemically, 2-ethoxy-3-[21-(1*H*-tetrazol-5-yl) biphenyl-4 ylmethyl]-3*H*-benzoimiadazole-4-carboxylic acid 1-cyclohexyloxycarbonyloxy ethyl ester[2] (Figure 1).

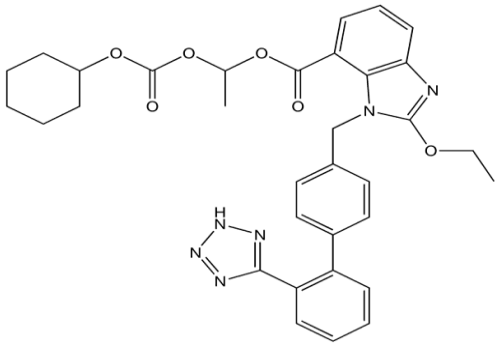

Figure 1: Structure of Candesartan cilexetil

Candesartan cilexetil is white to off-white crystalline powder having melting point of 157-160°C, and is water insoluble[3]. Candesartan acts by inhibits the binding of angiotensin II to the AT_1-Receptor. Candesartan cilexetil is hydrolyzed to candesartan during absorption from gastrointestinal tract[4]. It is used in the management of hypertension and may also be used in heart failure in patients with impaired left ventricular systolic function, either when ACE inhibitors are not tolerated, or in addition to ACE inhibitors[5]. Candesartan is highly bound to plasma proteins (>99%) and does not penetrate red blood cells. The protein binding is constant at Candesartan plasma concentration well above the range achieved with recommended doses[6]. Candesartan cilexetil is widely used for the treatment of hypertension and heart failure in clinical application[7-10]. It is available in 4 mg, 8 mg, 16 mg, 32 mg and can be used in the dose range of 8-32 mg/day[11,12].

Literature survey revealed that not much work has been done on the development of dissolution test for Candesartan cilexetil. Thus, it was thought to develop a simple, rapid, selective and reproducible reversed-phase high performance liquid chromatography (RP-HPLC) method for the determination of drug release of Candesartan cilexetil[13].

MATERIALS AND METHODS

Instrument

Dissolution experiments were performed using USP Dissolution Apparatus II (Distek, India) at the temperature of 37.0 ± 0.5ºC and the paddle speed of 50 rpm. A HPLC instrument is also used. The liquid chromatographic system consisted of Agilent Technology HPLC model containing pump, variable wave length programmable UV/Visible detector. Chromatographic analysis was performed using Kromacil C18 column with 150×4.6 mm internal diameter and 5 µm particle size. Mettler electronic balance was used for weighing purpose.

Reagents and Solvents

All the reagents and solvents were used of AR or HPLC grade (Made in Fisher Scientific Pvt. Ltd, RFCL Pvt. Ltd., Rankem, Merck Specialties Pvt. Ltd. Ranbaxy Lab. Ltd.). HPLC grade water was obtained by double distillation and purification through mille-Q water purification system (Made in Millipore Ltd, Bangalore). Potassium dihydrogen phosphate, tween 20 (0.15%, 0.25%, 0.35%) w/v, SLS (0.1%,0.25%,0.35%) w/v, pH 4.5 acetate buffer, pH 2.0 buffer, 0.1N HCl, pH 6.5 buffer, sodium hydroxide and phosphoric acid, hydrochloric acid, acetonitrile were used.

Methods

Preparation of reagents was done as per the US Pharmacopoeia 2011[14].

Preparation of phosphate buffer (pH 6.5)

Potassium dihydrogen phosphate (68 gm) and sodium hydroxide (8 gm) were dissolved in 10 L of distilled water. The pH of the solution was adjusted to 6.5 ± 0.05 with sodium hydroxide solution or phosphoric acid.

Preparation of 0.1 N HCl

Conc. HCl (85 mL) was dissolved in 10 L of water to get 0.1N HCl solution.

Preparation of buffer (pH 4.5)

Ammonium acetate (1.54 gm) was dissolved in 1L of water and the pH adjusted to 4.5±0.05 with glacial acetic acid. It was filtered through 0.45 µm or finer porosity membrane filter to get the clear solution.

Preparation of dissolution medium

The following dissolution medium was prepared:

- 0.1% w/v SLS was dissolved in 0.1N HCl.
- 0.1% w/v SLS was dissolved in pH 4.5 acetate buffer.
- 0.35% w/v polysorbate 20 (Tween 20) was dissolved in phosphate buffer of pH 6.5.
- 0.25% w/v SLS was dissolved in phosphate buffer of pH 6.5.

- 0.35% w/v SLS was dissolved in phosphate buffer of pH 6.5.
- 0.35% w/v Tween 20 was dissolved in phosphate buffer of pH 6.5.

Dissolution Parameters

Medium	:	Dissolution medium, 900 mL.
Apparatus	:	USP apparatus II (Paddle type)
Rotational speed	:	50 rpm
Temperature	:	37.0 ± 0.05^0C
Sampling interval	:	15, 30 and 45 min.

Preparation of mobile phase

Mobile phase was prepared by mixing a suitable quantity of buffer (pH 4.5) and acetonitrile in the ratio of 45:55. It was mixed well and then degassed.

Preparation of standard stock solution

A stock solution of Candesartan cilexetil was prepared by transferring 70 mg of Candesartan cilexetil to a 200 mL volumetric flask containing 20mL of acetonitrile. It was sonicated to dissolve the drug. The volume was made up with the dissolution medium.

Preparation of standard solution (for 32 mg Tablets)

Standard stock solution (5 mL) was diluted to 50 mL with the dissolution medium. It was filtered through 0.45 µm nylon filter.

Chromatographic Parameters:

Column	: Kromacil C18, 5 µm (150 mm x 4.6 mm)
Flow rate	: 1.5 mL/min
Detector	: UV at 257 nm
Column oven temperature	: 30^oC
Injection volume	: 50 µL
Run time	: 10 min

Various approaches have been suggested for designing the dissolution tests for poorly water soluble drugs. These include use of large volumes of dissolution medium, mixed organic-aqueous solvents, inclusion of surfactant, etc. In the present investigation, solubility of Candesartan cilexetil in distilled water and buffer solutions containing surfactant was assessed to prepare a dissolution medium that satisfied the sink conditions. In this study, solubility data was used as a basis for the development of dissolution medium for Candesartan cilexetil. The apparent solubility of Candesartan cilexetil in water, buffer solutions and buffer solutions with surfactant was determined at 37 °C.

Solubility Study

Candesartan cilexetil was placed in each of the flasks, having Teflon lined screw caps, containing 10 mL of the various solvents as shown in Table 1. The flasks were kept on a shaker incubator maintained at 37.0±0.5°C for 24 h. The resulting solutions were filtered through 0.45µm millipore filter and the filtrate was analyzed by HPLC against respective blank solutions. The solubility data as shown in Table 1 revealed that solubility of the drug was found to be highest in the medium containing 0.35% w/v Tween 20 in pH 6.5 Phosphate buffer (2.085 mg/mL). For establishing sink condition during dissolution test, the concentration on highest strength 32 mg in the selected media (900 mL of pH 6.5 Phosphate buffer with 0.35% w/v Tween 20) should be 3-10 times lower than the solubility of Candesartan cilexetil i.e 2.085 mg/mL.

For the highest dose of 32 mg, drugs are dissolved in 900 mL of dissolution media. The concentration achieved is 0.0355 mg/mL which is less than 1/3 to 1/10 times of solubility of Candesartan cilexetil, i.e (2.085/3= 0.695 mg/mL and 2.085/10= 0.2085 mg/ml)

Observation

Based on the above observations, it could be concluded that the proposed media was showing sink condition with respect to highest strength of Candesartan cilexetil tablets i.e 32 mg. It could be inferred from the results that the solubility of Candesartan cilexetil was independent of pH. Solubility increased in presence of surfactant. Since Candesartan cilexetil is water insoluble. We have tried to evaluate different media in pH range of 1.0-6.5 using different surfactants. 16 mg of the drug was added in 300 mL of media and observed for solubilization in pH 0.1N HCl, pH 4.5 acetate buffer and pH 6.5 phosphate buffer with surfactants like SLS and Tween 20 after sonication for 1 h. The observations are presented in the Table 2.

It was observed that drug got precipitated in some of the Media and the media became hazy. The drug was completely soluble in 0.1N HCl with 0.1% w/v SLS and 6.5 phosphate buffers with 0.35% w/v Tween 20, and the solution was found clear. pH 4.5 acetate buffers with 0.1% w/v SLS was also selected, since the media is less hazy and these 3 media (listed below) seemed to provide sink condition for dissolution of Candesartan cilexetil.

- 0.1N HCl with 0.1% w/v SLS
- 6.5 Phosphate buffer with 0.35% w/v Tween 20
- pH 4.5 Acetate buffer with 0.1% w/v SLS

The two formulations i.e Candesartan cilexetil Tablets INNOVATOR (Amias 32 mg) and SAMPLE (In-house batch, made in Ranbaxy Lab. Ltd. 32mg) of Candesartan cilexetil were used for this study.

Dissolution study

Dissolution experiments were performed using USP Dissolution Apparatus II (Distek, India) at a temperature of 37.0±0.5°C with the paddle speed of 50 rpm.

Preparation of Sample solution (For both Innovator and Sample)

One tablet was placed in each of six dissolution vessels and started the dissolution test. At the specific time interval (15, 30 and 45 min), 10 mL of aliquot was withdrawn from each dissolution vessel and replaced the volume with the same medium. The solution was filtered through 0.45 µm nylon filter and then analyzed using RP-HPLC method. Dissolution study was carried out with dissolution media selected on the basis of solubility data.

Trials (For Development)

For better dissolution rate of Candesartan cilexetil in biological system, selected dissolution medium from the solubility study were used:

- 0.1N HCl with 0.1% w/v SLS
- pH 4.5 Acetate buffer with 0.1% w/v SLS
- pH 6.5 Phosphate buffer with 0.35% w/v Tween 20.

These three types of dissolution media were used for the Innovator product. On the basis of % release of innovator (Figure 2), the pH 6.5 Phosphate buffer with 0.35% Tween 20 medium was selected.

For Sample of Candesartan cilexetil, the selected medium from innovator i.e pH 6.5 Phosphate buffer with 0.35% Tween 20 and pH 6.5 Phosphate buffer with other surfactant were also tried, which was as follows:

- pH 6.5 Phosphate buffer with 0.25% w/v SLS
- pH 6.5 Phosphate buffer with 0.35% w/v SLS
- pH 6.5 Phosphate buffer with 0.35% w/v Tween 20.

But In these trials, only the pH 6.5 Phosphate buffer with 0.35% Tween 20 shows the satisfactory result (Figure 3).

Chromatographic conditions

The mobile phase consisted of a mixture of pH 4.5 buffers and acetonitrile in the ratio of 45:55, mixed properly and then degassed. The flow rate of mobile phase was maintained at 1.5 mL/min

and detection wavelength was set at 257 nm with a run time of 10 min. The volume of injection was 50 µL. The Kromacil C18, 5 µm (150 mm x 4.6 mm) HPLC column was fitted, and temperature kept constant 30 ^{o}C on both sides of the column.

From the HPLC peak area, the % drug release was calculated by following formula:

$$\frac{\text{Area of test (sample)} \times \text{Dilution of std} \times \text{Potency} \times 100}{\text{Area of Std.} \times \text{Dilution Test (sample)} \times 100 \times \text{Label Claim}}$$

After calculating the % release of both Innovator (Amias 32mg) and Sample (In-house batch, Ranbaxy) of Candesartan cilexetil, it was found that the pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 showed satisfactory results (**Figure 2**). Other media did not show better results.

The data clearly indicated that drug release was completed in case of pH 6.5 Phosphate buffer with 0.35% w/v Tween 20. Based on these observations, the pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 could be a suitable dissolution medium for Candesartan cilexetil (**Figure 3**).

Similarity factor

Similarity factor was calculated for the % release of the drug in the selected medium i.e Phosphate buffer pH 6.5 with 0.35% w/v Tween 20. These are calculated between the Innovator (Amias 32 mg) and Sample (In-house batch, made in Ranbaxy Lab. Ltd.) of Candesartan cilexetil.

Similarity factor was found to be 73, by calculating the following formula:

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

Where,

n = No. of observations, f_2 = Similarity factor, w_t = Optional weight, R_t = % drug released (Innovator), T_t = % drug released (Sample). The dissolution similarity factor data was used to calculate the f_2 value Shah et al[15].

An f_2 value between 50 and 100 suggests that the two dissolution profiles are similar. So the sample of Candesartan cilexetil was found similar with the innovator (**Figure 4**).

Other Trial

Additional dissolution study was also performed to further support the above observations, and data generated by using varying concentration of sodium dodecyl sulphate (SLS) and Tween 20 in pH 6.5 Phosphate buffer. On the basis of % release of the drug, the results were found satisfactory only with pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 (**Figure 5**).

Therefore, pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 was selected for the better dissolution rate medium of Candesartan cilexetil

RESULTS AND DISSCUSSION

The selection of the medium was made on the basis of solubility data of Candesartan cilexetil in different dissolution medium at 37°C (**Table 1 & 2**). Drug release was carried out as per USP 2011 dissolution general specification at 50 rpm. All the buffers were made as per USP guidelines. The proposed dissolution was successfully applied for the better dissolution rate of Candesartan cilexetil in our body.

Table 1: Solubility studies of Candesartan cilexetil in different media at 37 °C.

S. No.	Solvent/Media	Saturation solubility (mg/mL)
1.	Water	0.005
2.	0.1N HCl (pH 1.2)	0.000
3.	pH 2.0 buffer	0.002
4.	pH 4.5 Acetate buffer	0.000
5.	pH 6.5 Phosphate buffer	0.000
6.	0.1N HCl+0.1% w/v SLS	0.132
7.	pH 4.5 Acetate buffer +0.1%w/v SLS	0.002
8.	pH 6.5 Phosphate buffer+0.1% w/v SLS	0.053
9.	pH 6.5 Phosphate buffer+0.25% w/v SLS	0.030
10.	pH 6.5 Phosphate buffer+0.35% w/v SLS	0.042
11.	pH 6.5 Phosphate buffer+0.15% w/v Tween 20	0.033
12.	pH 6.5 Phosphate buffer+0.25% w/v Tween 20	0.059
13.	pH 6.5 Phosphate buffer+0.35%w/v Tween 20	2.085

Table 2: Solubility observation of Candesartan cilexetil in different media.

Media	Solution
0.1N HCl with 0.05% w/v SLS	Slightly Hazy
0.1N HCl with 0.1% w/v SLS	Clear
0.1N HCl with 0.2% w/v SLS	Clear
0.1N HCl with 0.2% w/v Tween 20	Hazy
0.1N HCl with 0.35% w/v Tween 20	Hazy
pH 4.5 Acetate buffer with 0.05 % w/v SLS	Hazy
pH 4.5 Acetate buffer with 0.1 % w/v SLS	Less Hazy
pH 4.5 Acetate buffer with 0.2 % w/v SLS	Slightly Hazy
pH 4.5 Acetate buffer with 0.20 % w/v Tween 20	Hazy
pH 4.5 Acetate buffer with 0.35 % w/v Tween 20	Hazy
pH 6.5 Phosphate buffer with 0.05% w/v SLS	Hazy
pH 6.5 Phosphate buffer with 0.01% w/v SLS	Hazy
pH 6.5 Phosphate buffer with 0.2% w/v SLS	Hazy
pH 6.5 Phosphate buffer with 0.20% w/v Tween 20	Hazy
pH 6.5 Phosphate buffer with 0.35% w/v Tween 20	Clear

The data (trial) indicated that at 0.25% w/v concentration of Tween 20 in Phosphate buffer pH 6.5, incomplete drug release was observed, where as complete drug release observed with Tween 20 at 0.35% w/v concentration (**Figure 2 &3**). Therefore, the concentration of 0.35% w/v Tween 20 was selected for medium.

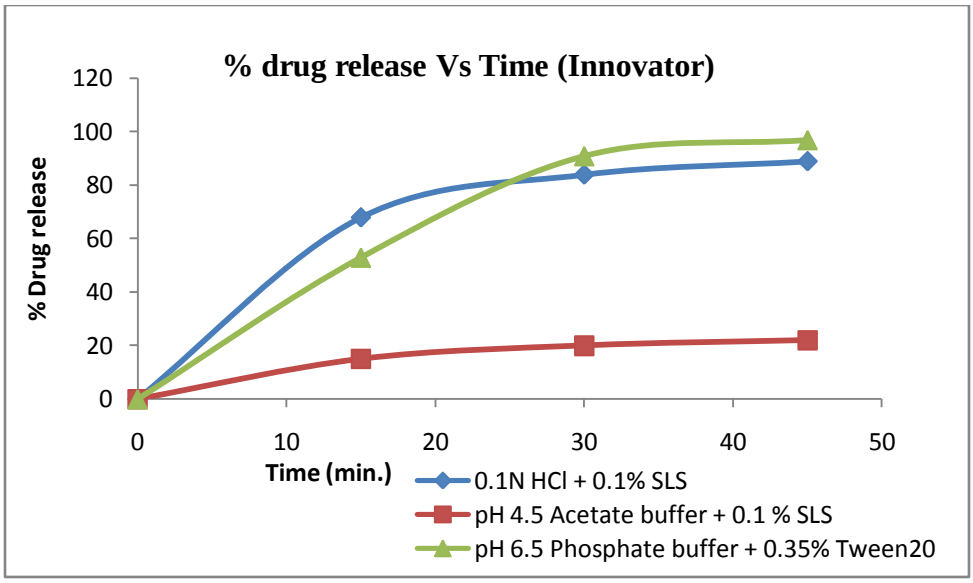

Figure 2: Graph showing % drug release from Innovator (Amias 32 mg)

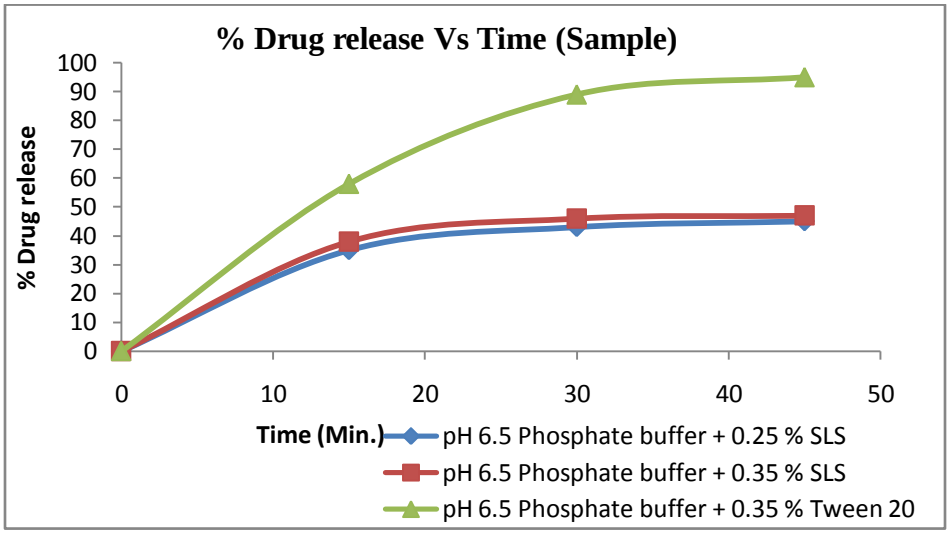

Figure 3: Graph showing % drug release from Sample (In-house batch, made in Ranbaxy)

Similarity factor was calculated for the % release of the drug in the selected medium i.e Phosphate buffer pH 6.5 with 0.35% w/v Tween 20. It was calculated between the Innovator (Amias 32 mg) and Sample (In-house batch, made in Ranbaxy Lab. Ltd.) of Candesartan cilexetil, and found to be 73. Similarity factor value between 50 and 100 suggests that the two dissolution profiles are similar. So the sample of Candesartan cilexetil was found similar with the innovator (**Figure 4**).

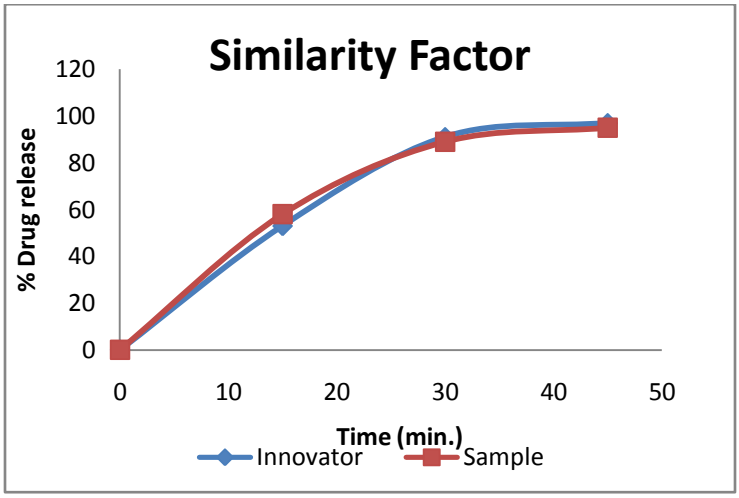

Figure 4: Graph showing similarity between Innovator and Sample

The data clearly indicated that at the same concentration of surfactant i.e 0.35% w/v SLS in Phosphate buffer pH 6.5, incomplete drug release was observed, where as complete drug release observed with 0.35% w/v Tween 20 (**Figure 5**). Hence, Tween 20 was preferred over SLS.

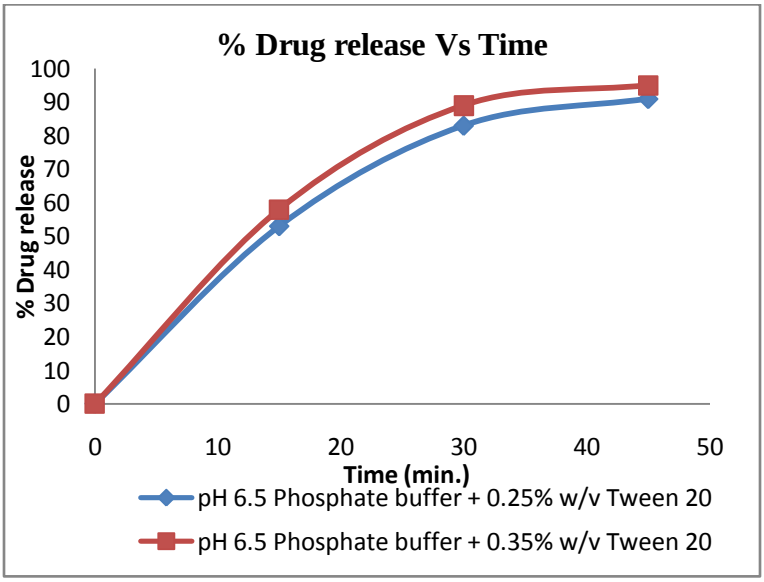

Figure 5: Graph showing % drug release of the drug

As 900 mL of pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 satisfied the sink condition, it was considered to be a suitable dissolution medium. The result indicated that the dissolution rate of Candesartan cilexetil increased with increase in Tween 20 concentration in the dissolution medium. Thus, pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 was selected for dissolution medium, because this media showed the satisfactory % release of the drug.

CONCLUSION

The performance of selected dissolution medium, pH 6.5 Phosphate buffer with 0.35% w/v Tween 20, was confirmed by conducting dissolution studies on two types of Candesartan

cilexetil tablets Innovator (Amias 32 mg) and Sample (In-House batch, made in Ranbaxy). It was found that the release of the drug was satisfactory in this medium. The results of the present study clearly indicated that 900 mL of pH 6.5 Phosphate buffer + 0.35% w/v Tween 20, as dissolution medium was suitable for in vitro dissolution testing of conventional Candesartan cilexetil formulations. This media showed better similarity factor of the sample of Candesartan cilexetil with that of the Innovator of this drug. Therefore, pH 6.5 Phosphate buffer with 0.35% w/v Tween 20 is the suitable dissolution media for Candesartan cilexetil.

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