



King Saud University  
Arabian Journal of Chemistry

[www.ksu.edu.sa](http://www.ksu.edu.sa)  
[www.sciencedirect.com](http://www.sciencedirect.com)



## ORIGINAL ARTICLE

# Development and validation of a dissolution test with reversed-phase high performance liquid chromatographic analysis for Candesartan cilexetil in tablet dosage forms



Vairappan Kamalakkannan <sup>a,b</sup>, Ayarivan Puratchikody <sup>b,\*</sup>,  
Lakshmanan Ramanathan <sup>c</sup>, Sundaravadivelu Jayapraba <sup>d</sup>

<sup>a</sup> Caplin Point Laboratories Limited, Puducherry 605 502, India

<sup>b</sup> Department of Pharmaceutical Technology, Anna University of Technology, Tiruchirappalli 620 024, India

<sup>c</sup> Dr. Reddy's Laboratoires Ltd., Hyderabad 500 016, India

<sup>d</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai 600 003, India

Received 25 June 2011; accepted 10 September 2011

Available online 17 September 2011

## KEYWORDS

*In vitro* release;  
Candesartan cilexetil;  
Validation;  
Reversed-phase high performance liquid chromatography

**Abstract** A simple, rapid, selective and reproducible reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the estimation of release of Candesartan cilexetil (CC) in tablets. Analysis was performed on an Agilent, Zorbax C<sub>8</sub> column (150 mm × 4.6 mm, 5 μm) with the mobile phase consisting of phosphate buffer (pH 2.5)–acetonitrile (15:85, v/v) at a flow rate of 1.0 mL/min. UV detection was performed at 215 nm and the retention time for CC was 2.2. The calibration curve was linear (correlation coefficient = 1.000) in the selected range of analyte. The optimized dissolution conditions include the USP apparatus 2 at a paddle rotation rate of 50 rpm and 900 mL of phosphate buffer (pH 7.2) with 0.03% of polysorbate 80 as dissolution medium, at 37.0 ± 0.5 °C. The method was validated for precision, linearity, specificity, accuracy, limit of quantitation and ruggedness. The system suitability parameters, such as theoretical plate, tailing factor and relative standard deviation (RSD) between six standard replicates were well within the limits. The stability result shows that the drug is stable in the

\* Corresponding author. Tel.: +91 9626609076, +91 431 2407995.

E-mail address: [puratchipharma@gmail.com](mailto:puratchipharma@gmail.com) (A. Puratchikody).

Peer review under responsibility of King Saud University.



prescribed dissolution medium. Three different batches (A, B and C) of the formulation containing 8 mg of Candesartan cilexetil was performed with the developed method and the results showed no significant differences among the batches.

© 2011 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

## 1. Introduction

Candesartan cilexetil (CC) is a commercially available antihypertensive prodrug containing one chiral center at the cyclohexyloxycarbonyloxy ethyl ester group (Ross and Papademetriou, 2004) (Fig. 1). In the gastrointestinal (GI) tract CC is rapidly absorbed and completely bioactivated by ester hydrolysis at the ester link, converted to active candesartan (McClellan and Goa, 1998). An important clinical application of CC is an angiotensin receptor blocker with insurmountable binding properties to the angiotensin-1 receptor, long duration of action and improved efficacy (Joost et al., 2011). Furthermore, it is a white to off-white crystalline powder with a molecular mass of 61 kDa. The solubility nature in benzyl alcohol is 0.3 M, and is insoluble in water ( $<8 \times 10^{-8}$  M). The partition coefficient ( $C_{\text{octanol}}/C_{\text{aqueous}}$ ) at pH 1.1, 6.9 and 8.9 is  $>1000$  indicating high hydrophobicity character (ATACAND<sup>®</sup>). It has a  $pK_a$  value of 6.0 (Cagigal et al., 2001).

Analytical method validation is a process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Food and Drugs Administration (FDA) regulations such as Good Manufacturing Practice (GMP), Good Laboratories Practice (GLP), Good Clinical Practice (GCP) and quality standards such as International Organization for Standardization (ISO17025) require analytical methods to be validated before and during routine use (Physicians' Desk Reference<sup>®</sup>, 2009). Method validation is defined as the process of proving through scientific studies that an analytical method is acceptable for its intended use. USP provides regulatory guidance for method validation (FDA, 2000) and recent guidelines for methods development and validation for new non-compendial test methods are provided by the FDA draft document (USP 25-NF 20, 2002).

Developing dissolution methods for poorly soluble compounds has been a consistent challenge for the pharmaceutical scientist. Because of inherently slow dissolution, poorly soluble compounds are good candidates for developing *in vitro* and *in vivo* correlations (IVIVCs) if intestinal permeability is high and drug dissolution is the controlling mechanism for the release of drug from the dosage form (ICH, 2000). Drug absorption from a dosage form after oral administration depends on

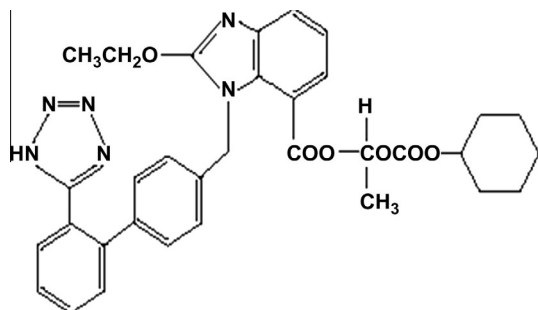


Figure 1 Chemical structure of Candesartan cilexetil.

the release of the drug from the pharmaceutical formulation, the dissolution and/or its solubilization under physiological conditions and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps *in vitro* dissolution may be relevant to the prediction of *in vivo* performance (Amidon et al., 1995; Emami, 2006).

Over the past decade several published literatures reported that there is no validated method for dissolution and moreover no official monograph was available for CC. Parameters to set up the dissolution test should be researched and defined for the drugs that do not possess official monographs (Emami, 2006). Importantly, the present work describes the development and validation of an accurate and reliable RP-HPLC method for the estimation of CC release in solid dosage form. The best dissolution conditions were used to evaluate the development and validation of a dissolution method and this method was used to evaluate the dissolution profile of three different batches of tablets.

## 2. Experimental

### 2.1. Chemicals and reagents

The pure drug CC was obtained as a free gift sample from Caplin point laboratories limited, Puducherry, India. Acetonitrile was purchased from Merck (India) Ltd. Sodium dihydrogen orthophosphate, phosphoric acid, potassium dihydrogen orthophosphate, polysorbate 80 and sodium hydroxide were procured from SD fine chemicals, Bangalore, India. All other reagents used were of analytical grade.

### 2.2. Instruments

USP type 2 rotating paddle apparatus (Electrolab, TDT-08L) was used to study the drug dissolution profile. The dissolution medium was deaerated by vacuum filtration and the temperature maintained at  $37.0 \pm 0.5$  °C throughout the study. Drug release estimation was performed using RP-HPLC equipped with agilent 1200 series quaternary pump (DE62974693) with a variable wavelength detector (DE71367145) at 215 nm and the pH of all solutions were determined by Hanna pH analyzer (microprocessor pH 211).

## 3. Method

### 3.1. Determination of solubility and dissolution optimization

Candesartan cilexetil solubility was determined using 900 mL of purified water, 0.1 N hydrochloric acid, acetate buffer (pH 1.2–2.2) and phosphate buffer (pH 4.5–7.2) with an amount of drug equivalent to three times of the dose in the pharmaceutical formulation (US Pharmacopoeia, 2007). Drug release was carried out as per USP dissolution general

specification at 50 rpm. Sampling aliquots of 10 mL were withdrawn at pre-determined time intervals (15, 30, 45 and 60 min), and replaced with an equal volume of dissolution medium to maintain a constant total volume of 900 mL. To assess the stability of CC in dissolution medium, samples were diluted by phosphate buffer (pH 7.2) with 0.03% polysorbate 80. The prepared solutions kept at different conditions, such as room temperature and at  $37.0 \pm 0.5$  °C for 24 and 2 h, respectively. The stability of these solutions was studied by comparing the values obtained with freshly prepared sample solutions.

### 3.2. Analytical method validation

RP-HPLC method was used to analyze the CC samples in phosphate buffer (pH 7.2). Validation was carried out for precision, linearity, specificity, accuracy, limit of quantitation and ruggedness according to US Pharmacopoeia (FDA, 1997) and International Conference on Harmonization (ICH) guideline (ICH (Q2R1), 2007). An isocratic HPLC analysis was performed on Agilent, Zorbax C<sub>8</sub> (150 × 4.6 mm, 5 μm) column maintained at ambient condition. Chromatographic separation was achieved with the mobile phase ratio of 15:85 (v/v) mixture of phosphate buffer (pH 2.5) and acetonitrile at a flow rate of 1.0 mL/min. Injection volume was 20 μL and the liquid chromatograph was equipped with variable wavelength detector at 215 nm.

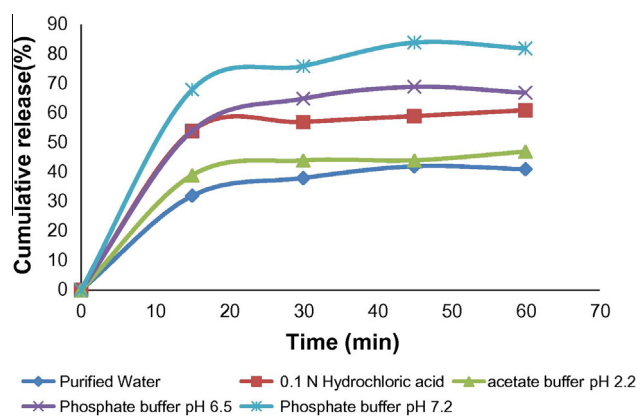
### 3.3. Evaluation of system suitability

Twenty microliters of standard solution was injected in triplicate before and after the analysis and the chromatograms were recorded. System suitability parameters like theoretical plate, tailing factor were also recorded. RSD of six replicates of standard was also taken. The column efficiency as determined from the active peak is not less than 6000 USP theoretical plates. USP tailing factor for the same peak is not more than 2.0 and RSD of six replicates of the standard solution is not more than 2.0%.

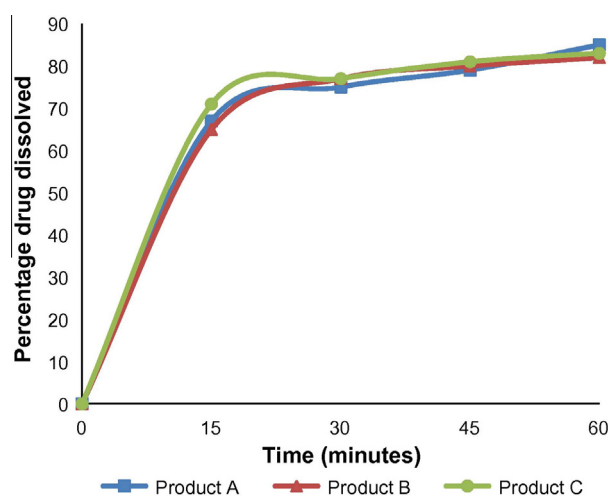
## 4. Results and discussion

### 4.1. Optimization of dissolution test conditions

The accomplishment of dissolution profile is recommended as a support in the development and optimization of drug formulation as well as in the establishment of *in vitro/in vivo* correlation. When dissolution test is not defined or if the monograph is not available, comparison of drug dissolution profiles is recommended on three different dissolution mediums (pH 1–7.5). *In vitro* dissolution was used to perform the release rate of drug products and to assure the quality of solid dosage forms by the pharmaceutical industry and regulatory agencies (ICH (Q2R1), 2007). The sink conditions are determined and expressed as a percentage of drug released. Purified water, 0.1 N hydrochloric acid, acetate buffer (pH 1.2–2.2) and phosphate buffer (pH 4.5–7.2) were used as dissolution medium and selected on the basis of solubility and screening study. From the above study the phosphate buffer (pH 7.2) provided highest drug release profile with greater stability, ensured excellent sink conditions and was selected as the best dissolution medium. The drug release profiles are shown in Fig. 2. Based on the solubility and screening study, phosphate buffer



**Figure 2** The cumulative release curves of Candesartan cilexetil from tablets at different medium.

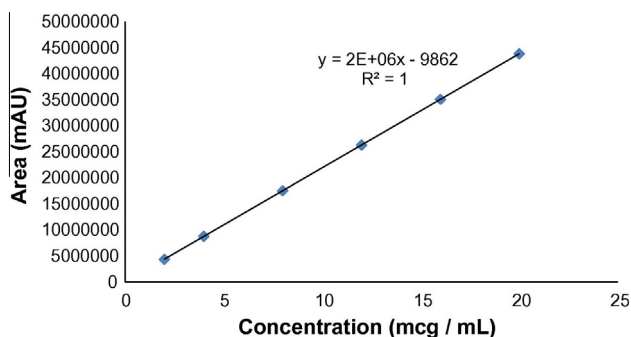


**Figure 3** In vitro release profile ( $n = 6$ ) of three batches of Candesartan cilexetil tablet.

(pH 7.2), was selected as the dissolution medium and USP type 2 rotating paddle apparatus at 50 rpm as an instrument. In these conditions, typical acceptance criteria for the amount of drug dissolved were in the range of 65–83%. In the present study, the percentage of drug released for all three different products were >80% in 60 min (Fig. 3) and the suggested acceptance criteria can be 75 (Q) in 60 min. The stability test indicated that CC is stable in the dissolution medium at room temperature and at  $37.0 \pm 0.5$  °C for 24 and 2 h, respectively. The results obtained from the initial and final response factors were within the acceptable range and not much difference between the stability and freshly prepared solutions.

### 4.2. Analytical method validation

In the current study, RP-HPLC method was used to determine the percentage drug release. HPLC is used to separate, identify and determine the concentration of a specific component in a mixture, moreover that this method is very fast, reproducible and easy to operate (Pharmacopoeial Forum, 2004). The developed method was validated to meet the requirements for a global regulatory filing. The validation parameters such



**Figure 4** Linearity graph of candesartan cilexetil.

**Table 1** Linear regression of Candesartan cilexetil.

Concentration (mcg/mL)	Area <sup>a</sup> (average)	Standard deviation	RSD
2	4,360,646.3	26,083	0.598
4	8,762,327.3	3492.8	0.04
8	17,528,692.7	7573.4	0.043
12	26,288,297.3	5351.3	0.02
16	35,063,016.3	19,091.7	0.054
20	43,809,040.7	4709.3	0.011

<sup>a</sup> Average of five determinations.

**Table 2** Calculation of regression line.

X (concentration in µg/mL)	Y (area obtained in mAU) <sup>a</sup>	XY	X <sup>2</sup>	Y <sup>2</sup>
2	4,360,646.3	8,721,292.6	4	1.90152E + 13
4	8,762,327.3	35,049,309.2	16	7.67784E + 13
8	17,528,692.7	140,229,541.6	64	3.07255E + 14
12	26,288,297.3	315,459,567.6	144	6.91075E + 14
16	35,063,016.3	561,008,260.8	256	1.22942E + 15
20	43,809,040.7	876,180,814.0	400	1.91923E + 15

<sup>a</sup> Average of five determinations.

as precision, linearity, specificity, accuracy, limit of quantitation and ruggedness were carried out in accordance with ICH and US Pharmacopoeia guidelines.

#### 4.2.1. Linearity

The linearity of CC response is evaluated from the range of 2.0–20.0 mcg/mL and showed a good correlation coefficient ( $r^2$ ) = 1.0. To validate linearity, the standard curve of CC was constructed by plotting concentration (mcg/mL) versus area response (mAU) which is shown in Fig. 4. The linear regression and slope were calculated and are shown in Tables 1–3.

#### 4.2.2. Specificity

Specificity is carried out with placebo solution and compared with the standard preparation. The drug release of CC in the dissolution medium was measured at 215 nm and the run time was extended up to 30 min. Two peaks were observed in the chromatogram, the major and minor peaks retention time was 2.2 and 1.3, respectively. The primary peak was due to polysorbate 80 and was confirmed by correlating with the blank

**Table 3** Slope calculation.

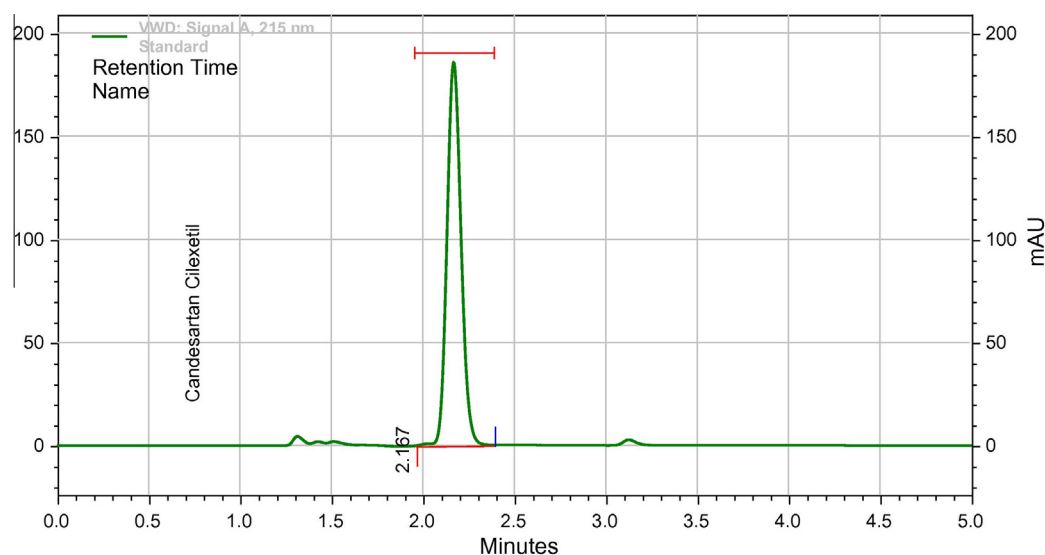
X axis	Y axis <sup>a</sup>	Y intercept	Slope
2	4,360,646.3	9862	2,185,254.2
4	8,762,327.3	9862	2,193,047.3
8	17,528,692.7	9862	2,192,319.3
12	26,288,297.3	9862	2,191,513.3
16	35,063,016.3	9862	2,192,054.9
20	43,809,040.7	9862	2,190,945.1

<sup>a</sup> Average of five determinations.

peak. The main peak was well separated from the blank peak and the resolution between these two peaks was more than 2 (Fig. 5). There were no other additional peaks observed which indicate no interferences by excipients and thus demonstrating that the proposed method is specific for the analysis of CC.

#### 4.2.3. Precision

The precision of an analytical procedure expresses the closeness of the agreement (degree of scatter) between a series of



**Figure 5** Standard chromatogram of Candesartan cilexetil.

**Table 4** Precision, intermediate and intraday precision of Candesartan cilexetil.

Replicates	Chemist-I (area) <sup>a</sup>	Chemist-II (area) <sup>a</sup>	Column-I (area) <sup>a</sup>	Column-II (area) <sup>a</sup>
Area-1	13,773,622	13,765,439	13,773,622	13,975,643
Area-2	13,991,726	13,756,393	13,991,726	13,759,234
Area-3	13,874,337	13,756,394	13,874,337	13,759,320
Area-4	13,705,374	13,850,324	13,705,374	13,856,290
Area-5	13,553,809	13,786,439	13,553,809	13,822,233
Area-6	13,571,089	13,745,633	13,571,089	13,944,752
Average	13,744,993	13,776,770	13,744,993	13,852,912
Standard deviation	171,341.9	38,554	171,341.9	91,635
RSD	1.247	0.2798	1.247	0.6615

<sup>a</sup> Average of five determinations.

measurements obtained from the multiple samples of the same homogeneous sample under the prescribed conditions. Repeatability is a measure of the precision under the same operating conditions over a short interval of time and it is also known as intra assay precision. A minimum six determinations at 100% of the standard concentration were tested to find out the average, standard deviation and related standard deviation, and all the calculated parameters were well within the prescribed limit. Intra-day precision and intermediate precision were done for ensuring the robustness of the method. The related standard deviation (RSD) of both the tests was well within the desirable limit of NMT 1.8% which is clearly indicated that the developed method is robust. Intraday and intermediate precision results are shown in Table 4.

#### 4.2.4. Accuracy

The accuracy of an analytical procedure is the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value. Accuracy is usually reported as percent recovery by an assay using the proposed analytical procedure of known amount of analyte added to the sample. The ICH also recommended assessing a

minimum of three determinations over a minimum of three concentration levels covering the specified range. The common method of determining accuracy is to apply the analytical procedure to the drug substance and to be quantitated against the reference standard of known purity. The range for the accuracy limit should be within the linear range. Typical accuracy of the recovery of the drug substance in the mixture is expected to be about 98–102%. Values of accuracy of the recovery data beyond this range are to be investigated. The precision concentration was 10 mcg/mL, hence the linearity range was selected from 7 to 14 mcg/mL. The known concentration of (20%, 40%, 60%, 80% and 100%) were added to the standard preparation (7 mcg/mL). The percentage recoveries obtained were considered under the acceptable range as per the ICH guidelines (Table 5).

#### 4.2.5. Limit of quantitation

Limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under stated experimental conditions. The quantitation limit is expressed as the concentration of analyte in the sample. The standard deviation and related standard deviation

**Table 5** Accuracy of Candesartan cilexetil.

Solution	Quantity added (known %)	Area <sup>a</sup>	Average area	Standard deviation	RSD	Recovery	Actual value	Accuracy
Soln-1	100 (7 mcg/mL)	9,629,430 9,629,583 9,705,621	9,654,878	43,944.8	0.455	–	–	–
Soln-2	120 (8.4 mcg/mL)	11,583,211 11,558,731 11,576,483	11,572,808	12,646.94	0.109	119.86	120	99.89
Soln-3	140 (9.8 mcg/mL)	13,588,943 13,756,329 13,889,445	13,744,906	150,576.3	1.096	142.36	140	101.69
Soln-4	160 (11.2 mcg/mL)	15,234,754 15,597,564 15,445,623	15,425,980	182,200.9	1.181	159.77	160	99.86
Soln-5	180 (12.6 mcg/mL)	17,123,893 17,326,740 17,590,870	17,347,168	234,157.7	1.350	179.67	180	99.82
Soln-6	200 (14 mcg/mL)	19,345,786 19,367,501 19,906,783	19,540,023	317,808.7	1.626	202.39	200	101.19

<sup>a</sup> Average of three determinations.

for the limit of quantitation were 15,686.2 and 1.1067%, respectively, which was well within the desirable limit of not more than 2.0%. The lowest quantifiable concentration was 1 mcg/mL and this parameter can be used for predicting the drug release in low dose formulation.

#### 4.2.6. Ruggedness

Intraday and intermediate precision were determined by analyzing the solutions by two different analysts, using different instruments, using multiple lots of column, in two different labs and on different days. The percentage RSD obtained under different conditions was below 2%. Table 4 represents the intermediate and intraday precision.

#### 4.3. System suitability

System suitability is an important parameter to ensure whether the used method was valid or not. The limit of theoretical plates and tailing factor was fixed as not less than 6000 and not more than 2, respectively. All the chromatograms theoretical plates were above 6000 and the tailing factor was less than 1. RSD results from six replicates showed adherence to the limits. The above results indicated that the developed method is valid and can be used for routine lab analysis.

## 5. Conclusion

The simple, sensitive and inexpensive isocratic RP-HPLC method was developed to determine the percentage drug release of CC tablets. The dissolution study showed that CC has good stability and the percentage drug released was satisfactory for all the evaluated batches from the formulation. The validation results show that the method is specific, accurate, linear, precise, rugged and robust. The run time is relatively short (5.0 min) which enables rapid quantification of many

samples in routine analysis. Therefore this method is proposed for the quality control studies of CC modified and conventional pharmaceutical dosage forms contributing to assure the therapeutic efficacy of the drug.

## References

- Amidon, G.L. et al., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. *Pharm. Res.* 12, 413–420.
- ATACAND®, 2010. Product monograph. <[http://www.astrazeneca.ca/documents/Product Portfolio/ATACAND, PM, en.pdf](http://www.astrazeneca.ca/documents/Product%20Portfolio/ATACAND,%20PM,%20en.pdf)> (accessed on 19.05.2010).
- Cagigal, E.L. et al., 2001. pK<sub>a</sub> determination of angiotensin II receptor antagonistic (ARA II) by spectrofluorimetry. *J. Pharm. Biomed. Anal.* 26, 477–486.
- Emami, J., 2006. *In vitro–in vivo* correlation: from theory to applications. *J. Pharm. Pharm. Sci.* 9, 169–189.
- FDA, 1997. Guidance for industry: dissolution testing of immediate release solid oral dosage forms. US Department of Health and Human Services, Centre for Drug Evaluation and Research (CDER).
- FDA, 2000. Analytical procedures and methods validation: chemistry, manufacturing, and controls. Federal Register (Notices) 65 (169), 52776–52777.
- ICH (Q2R1), 2007. Guideline on validation of analytical procedures: text and methodology. In: Proceedings of the International Conference on Harmonization, Geneva.
- ICH, 2000. Draft guidance on specifications: test procedures and acceptance criteria for new drug substances and products: chemical substances. Federal Register (Notices) 251, 83041–83063.
- Joost, Alexander et al., 2011. Candesartan cilexetil: an update. *Expert Opin. Pharmacother.* 12 (11), 1769–1780.
- McClellan, K.J., Goa, K.L., 1998. Candesartan cilexetil: a review of its use in essential hypertension. *Drugs* 56 (5), 847–869.
- Pharmacopoeial Forum, 2004. Pharmacopoeial Previews.

Physicians' Desk Reference® (PDR), 2009. 63rd ed.

Ross, Amy, Papademetriou, Vasilios, 2004. Candesartan cilexetil in cardiovascular disease. *Expert Rev. Cardiovasc. Ther.* 2 (6), 829–835.

United States Pharmacopoeia, 2007. 31st ed., United States Pharmacopoeial Convention Inc., Rockville, USA.

USP 25-NF 20, 2002. United States Pharmacopoeial Convention, Rockville, MD 2256.