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Analytical method development and validation for Candesartan Cilexetil as bulk drug and in pharmaceutical dosage forms by HPLC

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ABSTRACT

A simple, sensitive and inexpensive isocratic reverse-phase liquid chromatographic method has been developed for quantitative determination of Candesartan cilexetil, as bulk drug and in pharmaceutical dosage forms. Chromatographic separation was achieved on an Octa Decyl Silyl column (C-18, 250 mm x 4.6 mm, 5 µm particle) with a 20:80 (v/v) mixture of phosphate buffer, pH 2.5, and acetonitrile as mobile phase. The flow rate was 1.0 mL / min and the detection wavelength was 215 nm. Resolution of Candesartan cilexetil was greater than 2.0.The drug was subjected to forced degradation such as photochemical oxidation, chemical oxidation, acid hydrolysis, base hydrolysis, different pH range, aqueous & non aqueous solution and thermal stress. The substantial degradation occurred in alkaline and acidic media and under oxidative and hydrolytic stress conditions and also in aqueous & non-aqueous hydrolysis. The method was validated for Accuracy, Precision, Specificity, Limit of Detection, Limit of Quantification and Linearity Range.

Keywords: Candesartan cilexetil, Antihypertensive, Liquid Chromatography, Forced degradation, Validation.

INTRODUCTION

Candesartan is an antihypertensive drug commercially available as Cilexetil (cyclohexyl 1-hydroxy ethyl carbonate) ester form. It is a prodrug and is hydrolysed to Candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT_1 subtype angiotensin II

receptor antagonist. It is a non-peptide, chemically described as (±)-1-Hydroxyethyl 2-ethoxy-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl) benzyl]-7-benzimidazolecarboxylate, cyclohexyl carbonate (ester)

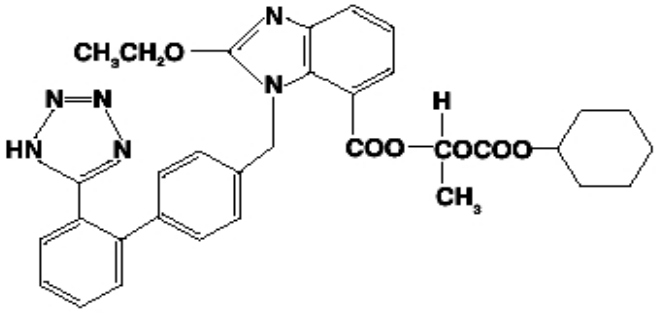


Fig 1 Structure Candesartan cilextil

Candesartan cilexetil is white to off-white crystalline powder with a molecular weight of 610.67. It is practically insoluble in water and sparingly soluble in methanol. Candesartan cilexetil is a racemic mixture containing one chiral center at the cyclohexyloxycarbonyloxy ethyl ester group. Following oral administration, Candesartan cilexetil undergoes hydrolysis at the ester link to form the active drug, Candesartan, which is achiral [1-3].

MATERIALS AND METHODS

2.1. Chemicals and Reagents

S.No	Ingredients	Source
1.	Candesartan cilexetil working Standard	Alembic Limited
2.	Sodium Dihydrogen Orthophosphate Orthophosphoric acid, Acetonitrile, Hydrogen Peroxide, Potassium Permanganate, Hydrochloric acid, Sodium hydroxide	Merk
3.	0.45µ Nylon Filter	Pall Life Sciences

2.2. Apparatus and Chromatographic condition

HPLC analysis for method development, forced degradation studies and method validation was performed with an Agilent 1200 series binary pump plus auto sampler and a photodiode-array detector (PDA). The output signal was monitored and processed using Chemstation software resident in a Pentium computer (Digital Equipment). Chromatographic separation was achieved on an Octa Decyl Silyl column (C-18, 250 mm x 4.6 mm, 5 µm particle) with a 20:80 (v/v) mixture of phosphate buffer, pH 2.5, and acetonitrile as mobile phase. The flow rate was 1.0 mL / min[4-6].

2.3. Preparation of Solutions

2.3.1. Buffer Preparation:

1.38 g of Sodium Dihydrogen Orthophosphate was weighed and dissolved in 1000ml of water and then the pH was adjusted to 2.5 with Orthophosphoric acid.

2.3.2. Mobile Phase:

A mixture of buffer and acetonitrile in the ratio of 20:80 was used as mobile phase.

2.3.3. Standard Preparation:
A stock solution of Candesartan cilexetil (0.5 mg / ml) was prepared by adding about 50mg of Candesartan cilexetil working standard into a 100ml volumetric flask and the volume was made up to 100 ml with mobile phase. 5ml of this solution was pipetted into a 50ml volumetric flask and the volume was made with mobile phase.

2.3.4. Sample Preparation:
50mg of Candesartan cilexetil working standard was weighed and transferred into a 100ml volumetric flask, 30ml of mobile phase was added to dissolve the contents and the volume was made with the same solvent. 5ml of this solution was taken and added in to 50 ml volumetric flask and the volume was made with mobile phase.

Method Validation
3.1. Validation of candesartan cilexetil method of analysis [7-9]
3.1.1. Accuracy
The accuracy of an analytical procedure is the closeness of the test result obtained by the procedure to the true value. Accuracy was calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals.

Procedure
Separately the solutions were injected and details were given below.

Preparation of Standard stock solution
50mg of Candesartan cilexetil working standard was weighed and dissolved in 100ml volumetric flask and the volume was made up to the mark with mobile phase.

Preparation of Solution A
5mL of the standard stock solution was diluted to 50mL in a 50ml standard flask with the same solvent.

Preparation of Solutions B,C & D
5mL of the Solution A was taken in a 50ml standard flask and spiked with 20% of known concentration and the volume was made up to the mark with the same solvent and this solution was named as Solution B. Same way solutions C and D were prepared by taking 5mL of the solution A in a 50ml standard flask and spiking with 40% and 60% of known concentration respectively. The results were given in results and discussion section.

3.1.2. Precision
The precision of an analytical procedure is the degree of agreement among the individual test result when the procedure is applied repeatedly to multiple sampling of a homogeneous sample. The precision of an analytical procedure is usually expressed as the standard deviation or related standard deviation (co-efficient of variation) of serious of measurements. Precision may be a measure of either a degree of reproducibility or of repeatability of the analytical procedure under normal operating conditions. The ICH documents recommend that using a minimum of six determinations at 100% of the test concentration.

Procedure

Standard solution

100mg of Candesartan cilexetil working standard was taken in a 100ml volumetric flask, dissolved and the volume made up to the mark with mobile phase. 5mL of the resulting solution was diluted to 50ml in a 50mL standard flask with the same solvent. The above solution was injected separately and six replicates were taken. Co-efficient of variation was calculated (RSD). The results were given in results and discussion section.

3.1.3. Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. These comparisons should include samples stored under relevant stress conditions (Example: light, heat, humidity, acid/base hydrolysis and oxidation).

Photochemical oxidation

The photochemical oxidation can be carried out by using hydrogen peroxide as catalyst. 50mg of Candesartan cilexetil working standard was taken in a 100ml volumetric flask, dissolved and the volume made up to the mark with 30% solution of Hydrogen Peroxide**.** This solution was maintained at 60°C for 24 hours, filtered through nylon filter paper of pore size 0.45µm and injected.

Chemical oxidation

The chemical oxidation can be carried out by using potassium permanganate as catalyst. 50mg of Candesartan cilexetil working standard was taken in a 100ml volumetric flask, dissolved and the volume was made up to the mark with 0.1M solution of Potassium Permanganate**.** This solution was maintained at 60°C for 24 hours, filtered through nylon filter paper of pore size 0.45µm and injected.

Acid hydrolysis

The acid hydrolysis can be carried out by using hydrochloric acid (1M). 50mg of Candesartan cilexetil working standard was taken in a 100ml volumetric flask, dissolved and the volume was made up to the mark with 1M solution of Hydrochloric acid**.** This solution was maintained at 60°C for 24 hours and then filtered through nylon filter paper of pore size 0.45µm and injected.

Base hydrolysis

The base hydrolysis can be carried out by using sodium hydroxide (1M) as catalyst. 50mg of Candesartan cilexetil working standard was taken in a 100ml volumetric flask, dissolved and the volume was made up to the mark with 1M solution of Sodium hydroxide**.** This solution was maintained at 60°C for 24 hours and then filtered through nylon filter paper of pore size 0.45µm and injected. The results were given in results and discussion section.

3.1.4. Limit of detection

It is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. Thus, limit tests merely substantiate that the amount of analyte is above or below a certain level. The detection limit is usually expressed as the concentration of analyte (e.g., percentage, parts per billion) in the sample. The minimum

concentration at which the analyte can reliably be detected is established. Typically acceptable signal-to-noise ratios are 2: 1 or 3: 1. Other approaches depend on the determination of the slope of the calibration curve and the standard deviation of responses.

Procedure
Limit of detection was carried out with 0.5mcg or 500ppb solution.

Preparation of 500ppb solution
50mg of the Candesartan cilexetil working standard was taken in a 50ml standard flask, dissolved and the volume was made with mobile phase. To 5ml of the previous solution in to 50ml volumetric flask, made up to volume with mobile phase. To 5ml of the previous solution in to 50ml volumetric flask made up to volume with mobile phase. To 5ml of the previous solution in to 100ml volumetric flask made up to volume with mobile phase. Separately 20µl solution was injected and the height of signal peak (S) and noise peak (N) was calculated. The results were given in results and discussion section.

3.1.5. Quantification Limit
The quantification limit is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The quantitation limit is expressed as the concentration of analyte. In the case of instrumental analytical procedures that exhibits background noise. Quantification limit will normally higher.

Preparation of Standard stock solution: (for Quantification limit)
50mg Candesartan Cilexetil standard was taken in 100ml volumetric flask, dissolved and the volume was made with mobile phase. To 5mL of previous solution in 50mL volumetric flask, made volume with mobile phase.

Sample solutions
1ml, 2ml, 3ml, 4ml & 5ml of the standard stock solution were taken in four 50ml volumetric flasks and the volume was made with mobile phase to prepare 1mcg/mL, 2mcg/mL, 3mcg/mL, 4mcg/mL & 5mcg/mL solutions respectively. The results were given in results and discussion section.

3.1.6. Linearity and range
Linearity of an analytical procedure is its ability to elicit test results that are directly, well defined mathematical transformation, proportional to the concentration of analyte in samples within the given range. The range of the analytical procedure is the interval between the upper and lower levels of the analyte (including these levels) that have been demonstrated to be determined with a suitable level of precision, accuracy, linearity using the procedure as written. The range is normally expressed in the same units as test result (E.g.: percent, part per million obtained by the analytical procedure).

Procedure
The concentration of the analyte was given in the X- axis and corresponding in Y- axis.

Standard stock solution: (for Linearity range)
50mg Candesartan cilexetil standard was taken in 100ml volumetric flask, dissolved and the volume was made upto the mark with mobile phase.

Sample solutions:
2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml of standard stock solutions were taken separately in seven 50ml volumetric flasks and the volume was adjusted with mobile phase to prepare 20mcg/mL, 30mcg/mL, 40mcg/mL, 50mcg/mL, 60mcg/mL, 70mcg/mL, 80mcg/mL solutions respectively. The results were given in results and discussion section.

RESULTS AND DISCUSSION

4.1. Accuracy:

The average of 6 replicates of **Solution A** was the known concentration of the 100%. The average of 6 replicates of **Solution A** was 5282.03. The results showed that the present method was accurate when analyzed for its recovery against the performed Range.

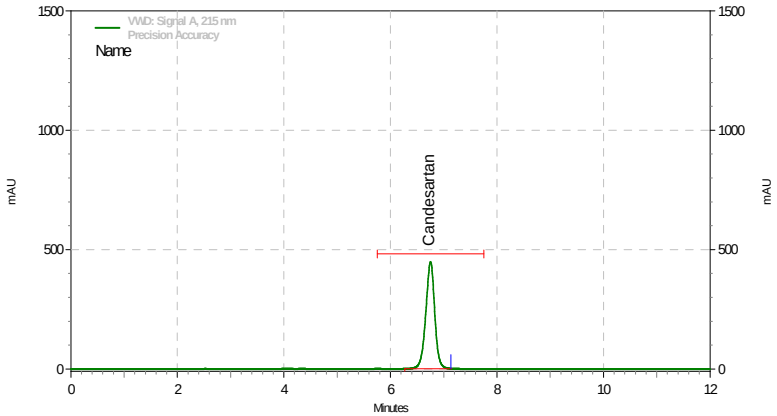
Table 1: reports of accuracy

Solutions	Quantity added (Known)	Area (obtained)	Average	Recovery (in %)	Accuracy
Solution B	20%	6392.69	6391.557	121.006%	100.84%
		6391.91			
		6390.07			
Solution C	40%	7469.42	7499.083	141.97%	101.41%
		7462.31			
		7565.52			
Solution D	60%	8565.7	8561.053	162.08%	101.30%

HPLC Chromatograms

Fig. 3 :Accuracy

Flow Rate: *1.0 mL/min*
Vial: *2*
Inj volume: *20 uL*
Sample Info: *Precision Accuracy*
Column: *C18, 250X4.5mm 5um*



4.2. Precision

The present method was validated for its precision. The RSD (Relative Standard Deviation) of the standard area was within the Limit. RSD: 1.408%[Limit: NMT: 2%]

Table 2: Results Of Precision

X – axis	Y – axis
Area – 1	5246.00
Area – 2	5172.76
Area – 3	5238.13
Area – 4	5325.81
Area – 5	5357.11
Area – 6	5352.36
Average	5282.028333
Std deviation	74.38455147
RSD%	1.408257335

Fig.4:Low range precision accuracy

Flow Rate: 1.0 mL/min
Vial: 71
Inj volume: 20 uL
Sample Info: LRP Accuracy 10mcg
Column : C18, 250X4.5mm 5um

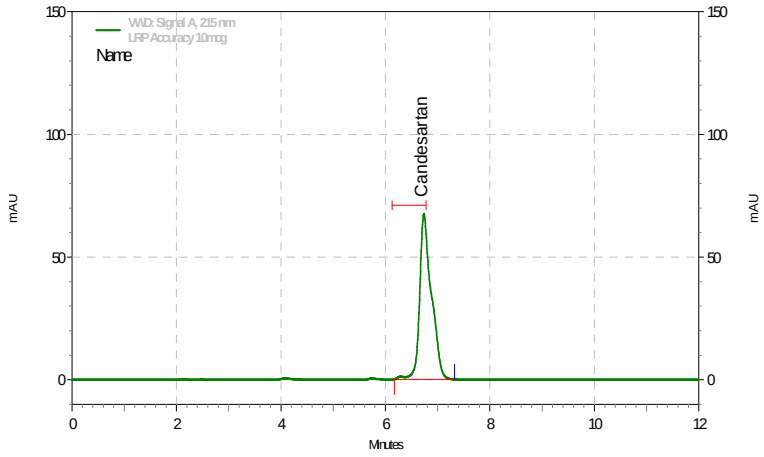


Fig. 5:High range precision accuracy

Flow Rate: 1.0 mL/min
Vial: 11
Inj volume: 20 uL
Sample Info: HRP Accuracy(100mcg)
Column : C18, 250X4.5mm 5um

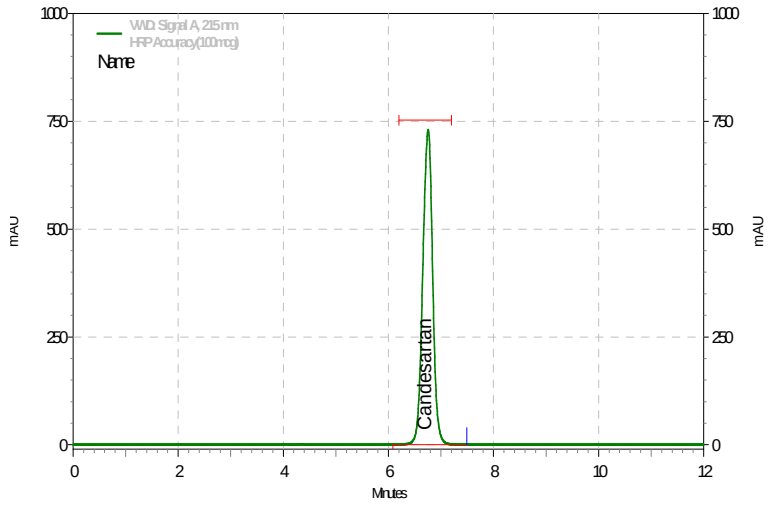
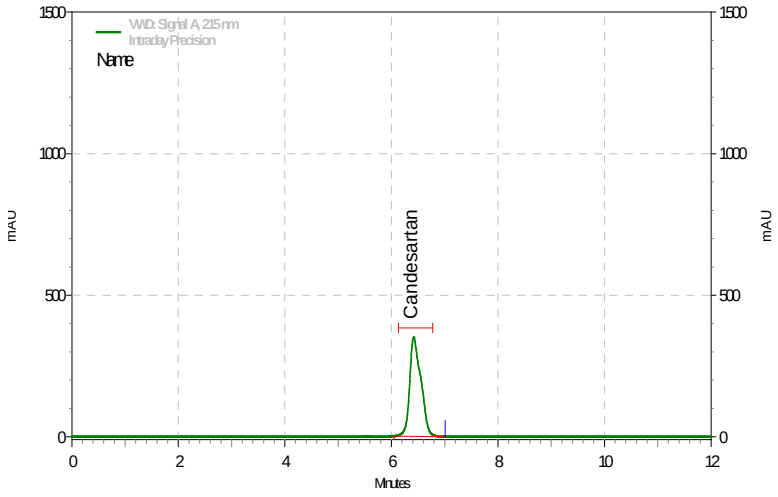


Fig. 6:Intraday Precision

Flow Rate: 1.0 mL/min
Vial: 51
Inj volume: 20 uL
Sample Info: Intraday Precision
Column : C18, 250X4.5mm 5um



4.3. Specificity

Exposing to various stress condition has yielded numerous degradation products. Peaks of such degradative products observed by the present method. Hence the method was specific for detecting the active and its degradants.

4.4. Limit of detection:

The ratio of signal to noise (S/N) was within the Limit of 2: 1 or 3: 1.
Height of signal (500ppb solution) = 4.2
Height of Noise (500ppb solution) = 0.61

Inference: The present method was validated for its Limit of Detection. The ratio of signal to noise (S/N) was within the Limit.

4.5. Quantification limit:

The limit of quantification was performed from 1mcg to 5mcg. It obeys as per the Ordinates plotted in the graph.

Fig.7:Limit of quantification

Flow Rate: 1.0 mL/min
Vial: 56
Inj volume: 20 uL
Sample Info: LOQ
Column : C18, 250X4.5mm 5um

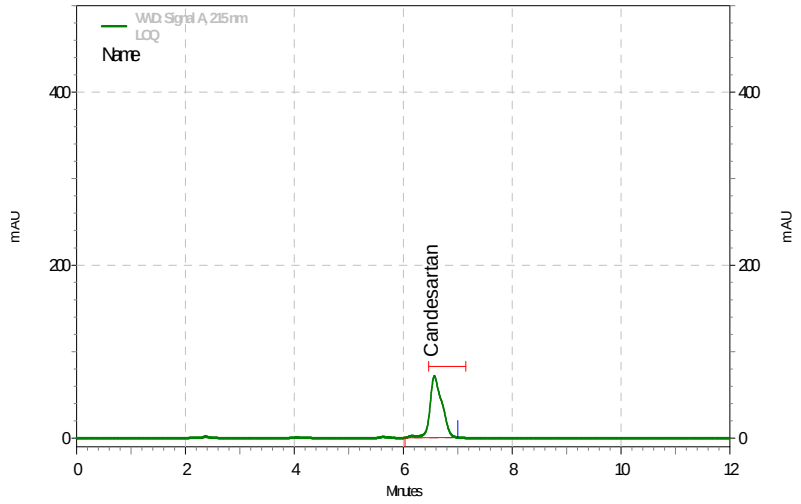
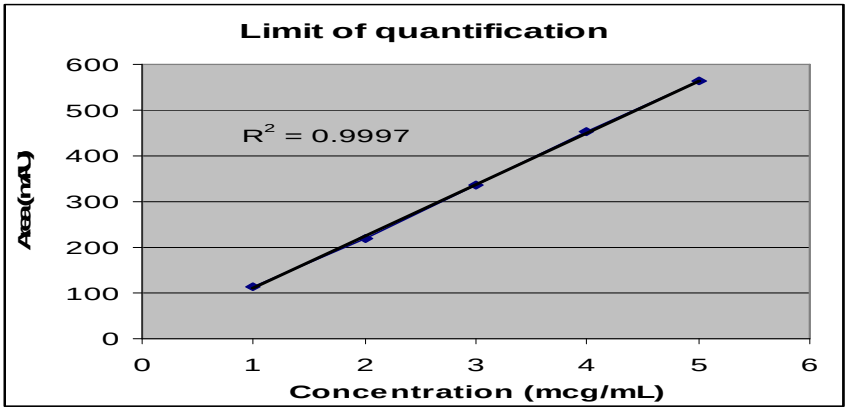


FIG.8: Limit of quantification



4.6. Linearity and range

The concentration was plotted in the graph (**Figure2)** which pertains a linear line and hence the linearity range was from 20mcg to 80mcg.

Table.3: limit of quantification

Concentration (mcg/ml)	Area (mAU)
1	113.32
2	219.25
3	335.50
4	452.84
5	564.27

Table 4: reports of linearity

X – axis	Y- axis
mcg/Ml	(Area) mAU
20	2171.08
30	3289.77
40	4380.52
50	5357.16
60	6387.26
70	7475.08
80	8575.09

Fig. 9:Linearity

Flow Rate: 1.0 mL/min
Vial: 61
Inj volume: 20 uL
Sample Info: Linearity 10mcg
Column : C18, 250X4.5mm 5um

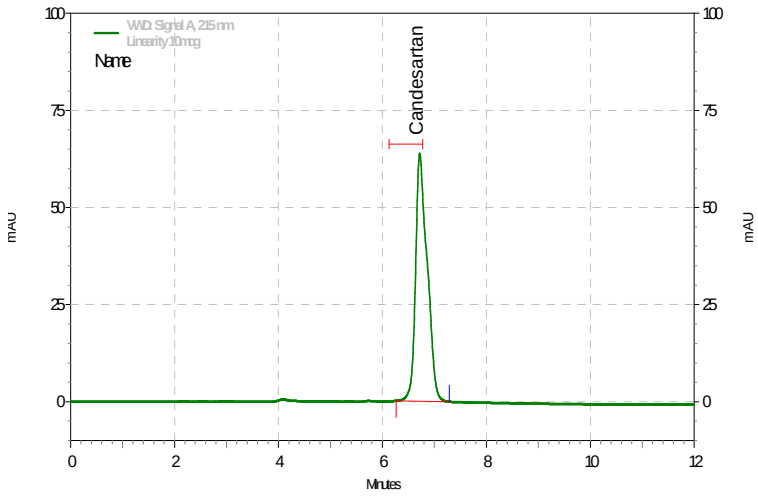
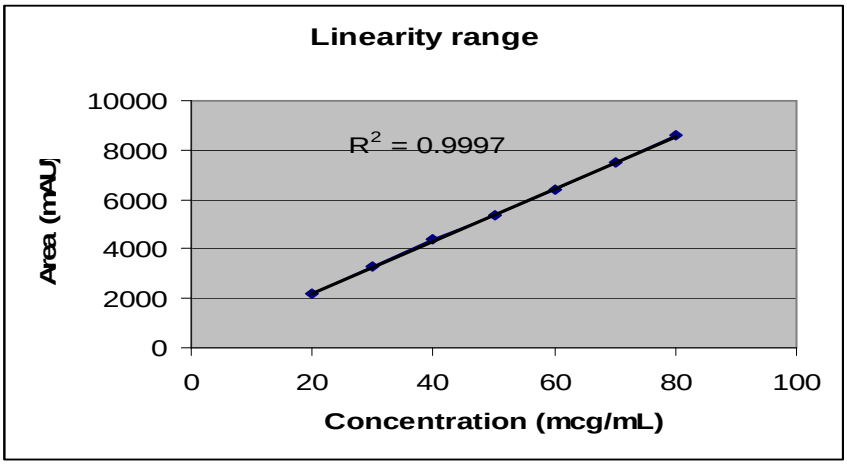


Fig.10: linearity and range



CONCLUSION

A simple and accurate reversed-phase HPLC method has been developed for determination of Candesartan Cilexetil. The method was validated by testing accuracy, precision, limits of detection, limit of quantification, specificity and linearity Range. The Retention Time (RT) was 7.3 minutes which enables its application for routine analysis of Candesartan Cilexetil in bulk drug analysis as well as in pharmaceutical dosage forms.

REFERENCES

[1] The United States Pharmacopoeia, 29/NF 24. The official compendia of standard Asian Edition, **2006**, 3050-3053
[2] British pharmacopoeia, volume IV, the British pharmacopoeia commission, London, **2005**. A-167
[3] The Merk Index, An encyclopedia of Chemicals, Drugs and Biologicals – 13th edition
[4] P.D.Sethi, High performance thin layer chromatography, first edition,CBS publisher and distributor, New Delhi,1st edition, **1996**, 4-28.
[5] Sagar B. Wankhede, Nitin R. Dixit and Sohan S. Chitlange, *Der Pharmacia Lettre*, **2011**, 3(1): 1-7
[6] Rashid R Munjewar, Mazahar Farooqui and Sayyed Husain, *Der Pharmacia Lettre*, **2010**, 2(6): 244-251
[7] International Conference on Harmonization ICH guidelines on validation of analytical procedures: text and methodology Q2 (R1): FDA. Federal Register **1995,** 60:11260
[8] N.V. Ramakrishna, K.N. Vishwottam, S. Manoj, M. Koteshwara, S. Wishu, and D.P. Varma, *Biomed. Chromatogr*., 19, 751 (**2005**)
[9] Thomson M.; Ellison S.L.R.; and Wood R. *Pure Appl. Chem* **2002** 74(50) 835-855