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Method Development and Validation of Candesartan by RP-HPLC

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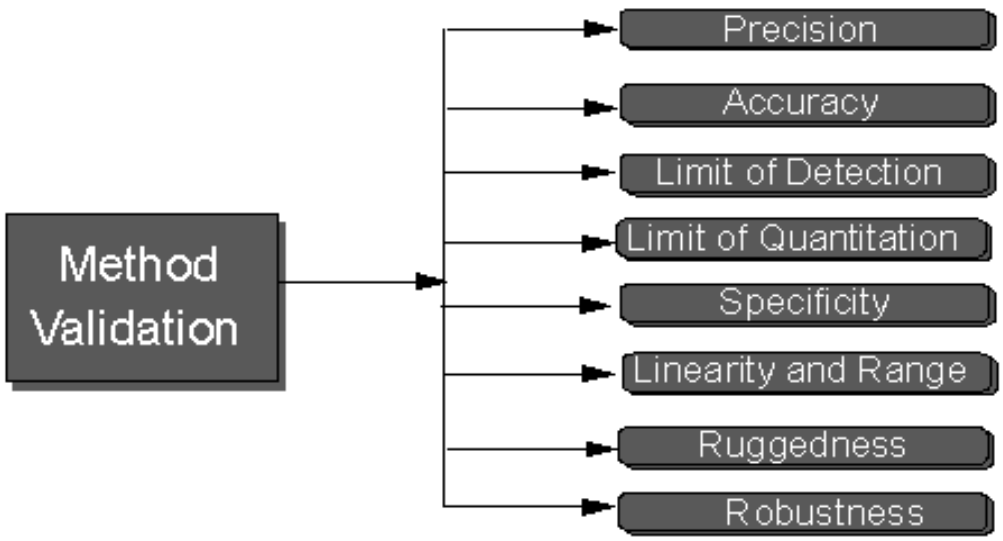
ABSTRACT

A simple, sensitive, rapid and selective isocratic reversed phase High Performance Liquid Chromatographic (HPLC) method has been developed for estimation of Candesartan from bulk drug dosage from using a mobile phase consisting mixture of Acetonitrile : $KH2PO_4$ Buffer (pH 2.8) (80:20 v/v) Composition of buffer: (0.272gm in 200ml HPLC water and pH adjusted to 2.8 using orthophosphoric acid) at the flow rate of 1.2mL/min using cosmosil C_{18} (250 cm x 4.6 mm, 5 µm) column as stationary phase. The retention time of Candesartan found to be 6.39 min. The eluent was detected at 230 nm. Linearity was observed in the concentration range of 100-180 ppm for Candesartan. Percent recoveries obtained for Candesartan were 96.33% . The correlation coefficient for Candesartan was found to be 0.995. After performing analysis by different analysts, it was found that the RP-HPLC method for the determination of Candesartan was found to be Rugged. Percent RSD for robustness was well within the acceptable USP limits, ensuring that the proposed method was robust. For candesartan the LOD were found to be 0.095 µg/ml and the LOQ were found to be 0.58 µg/ml. This demonstrated that the developed RP-HPLC method was simple, linear, precise, accurate, robust, and rugged, could be conveniently adopted for the routine quality control analysis of Candesartan, from its pharmaceutical formulations and bulk drug. Developed method was found to precise, accurate and validated as per pharmacopeial standard.

INTRODUCTION

VALIDATION OF ANALYTICAL METHODS [1-12]:

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications[1]. The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use has developed a text on the validation of analytical procedures[2]. The United States Food and Drug Administration (USFDA) have proposed guidelines on submitting samples and analytical data for methods validation[3-5]. The United States Pharmacopoeia (USP) has published specific guidelines for method validation for compound evaluation[6].



USP/ ICH Method Validation Parameters

Method needs to be validated or revalidated[13-14]:

- Before their introduction into routine use.
- Whenever the conditions changes for which the method has been validated. E.g. Instrument with different characteristics.
- Whenever the method is changed, and the change is outside the original scope of the method.

Typical validation characteristics which should be considered are as follows:

- Accuracy

- Precision
- Repeatability
- Intermediate precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range

MATERIALS AND METHODS

MATERIALS

1. **Drug sample suppliers & Manufacturer:**

Sr. No.	Name of Drugs	Drug supplies & Manufacturer.
1	Candesartan	Macleods Pharmaceuticals (Mumbai), Maharashtra,India.

2. **Instrument used:**

Equipment	Company
Maxia220 electronic balance	Shinko Denshi Co Ltd, Japan
UV 150-02 ,Visible double beam spectrophotometer	Shimadzu corporation ,Japan
Digital P^H Meter	Global , Ltd. Model No:-PGB100
Sonicator	Wenser
HPLC Binary Gradient System Model No:-3000 series HPLC pump : LC-P-4000 Column: Cosmosil C-18 (4.6mm×250mm,Partical size 5µm) UV-VIS detector	Analytical Technologies Ltd.
Analytical Balance Model No. PGB 100	Wenser

3. List of Reagents and Chemicals used:

Name of chemicals	Suppliers
Double distilled Water	RAP Analytical Lab, Nasik.
Potassium Di hydrogen Phosphate	Research Lab Fine Chem Industries.
Ortho Phosphoric acid	Research Lab Fine Chem Industries, Mumbai.
Acetonitrile HPLC grade	MerkSpecialitiesPvt Ltd, Mumbai.

Software used:

- HPLC Workstation.

METHODS:

1. Selection of analytical wavelength:

1.1 Standard stock solution of Candesartan:

10mg of Candesartan were accurately weighed, transferred to separate 10 ml volumetric flasks, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Candesartan. 1 ml of above solution transferred to 10 ml volumetric flask and the volume was made with diluents. The concentration of Candesartan is 100µg/ml.

1.2 Determination of λmax of Candesartan:

Standard stock solution of Candesartan was diluted separately with diluents to obtain final concentration of 10 µg/ml. Solution was scanned using UV-Visible Spectrophotometer in the spectrum mode between the wavelength range of 400 nm to 200 nm.

1.3 Determination of Absorption maxima:

By appropriate dilution of standard drug solutions with acetonitrile, solutions containing 10 µg/ml Candesartan were scanned separately in the range of 400-200nm to determine the wavelength of maximum absorption for the drugs.

2. HPLC method development:

2.1 Selection of Mobile phase:

Candesartan was injected into the HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation. After several permutation and combination, it was found that mixture of Acetonitrile : Buffer, with orthophosphoric acid to adjust the pH p^H,gives satisfactory results as compared to other mobile phases. Finally, the optimal composition of the mobile phase contains about 80 volume of Acetonitrile and 20 volume of Buffer [pH 2.8], as it gave high resolution of Candesartan with minimal tailing.

2.2 Preparation of mobile phase:

2.3 Preparation of Buffer:

An accurately weighed quantity of about 0.272gm of Potassium Dihydrogenortho Phosphate was taken in 500 ml volumetric flask dissolved in sufficient quantity of HPLC water, then sonicated for 15 min and diluted to 200ml with the HPLC water. Then adjust the pH up to 2.8 with orthophosphoric acid and filter through a 0.45µm membrane filter., gives the formation of buffer.

2.4 Mobile phase

Finally, the optimal composition of the mobile phase contains about 20 volume of buffer and 80 volume of Acetonitrile. [Acetonitrile : KH_2PO_4 Buffer(80:20)]

2.5 Preparation of standard stock solution:

10 mg of Candesartan were accurately weighed, transferred to separate 10 ml volumetric flasks, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Candesartan. 1 ml of above solution transferred to 10 ml volumetric flask and the volume was made with diluents. The concentration of Candesartan is 100µg/ml.

2.6 Loading of mobile phase:

Filtered & degassed mobile phase was loaded in the reservoir. Priming was done for each freshly prepared mobile phase.

2.7 Baseline stabilization:

The detector was turned on for an hour before the actual run in order to obtain the stable UV light. The mobile phase run was started at desired flow rate & the run was continued until the stable baseline was obtained.

2.8 Loading of samples:

Well prepared & filtered samples of Candesartan were loaded into the Rheodyne injector port using a syringe & the sample was then injected.

2.9 Washing the column:

Once the analysis of samples was finished, the column was first washed by flushing with the mobile phase for half an hour.

2.10 Chromatographic conditions:

Following are the optimized chromatographic condition for RP-HPLC method

Parameters	**Values**
Column	cosmosil C_{18}
Wavelength	230nm
Flow rate	1.2ml/min
Injection volume	20µl
Temperature	Ambient
Runtime	10 min

2.11 Assay of Candesartan:

10mg Candesartan were accurately weighed, transferred to separate 10 ml volumetric flasks, dissolved in the mobile phase and dilute to volume with the same solvent mixture to furnish stock solutions containing 1000µg/ml of Candesartan. From this solution, appropriate dilutions of Candesartan were made to get the final concentrations and finally the solutions were filtered through Whatman filter paper. A 20 µl sample was injected under optimized chromatographic conditions. The peak areas were measured at 230 nm and the percent purity and %RSD was calculated.

RESULTS AND DISCUSSION

The results of Method Development and Validation of Candesartan including all the analytical data with chromatograms were given as follows:

1. UV ANALYSIS FOR DETECTION OF WAVELENGTH:

1.1. Determination of λmax of Candesartan:

The standard solution of Candesartan was scanned at different concentrations in the range of 200-400nm and the λmax was found to be 230 nm against reagent blank.

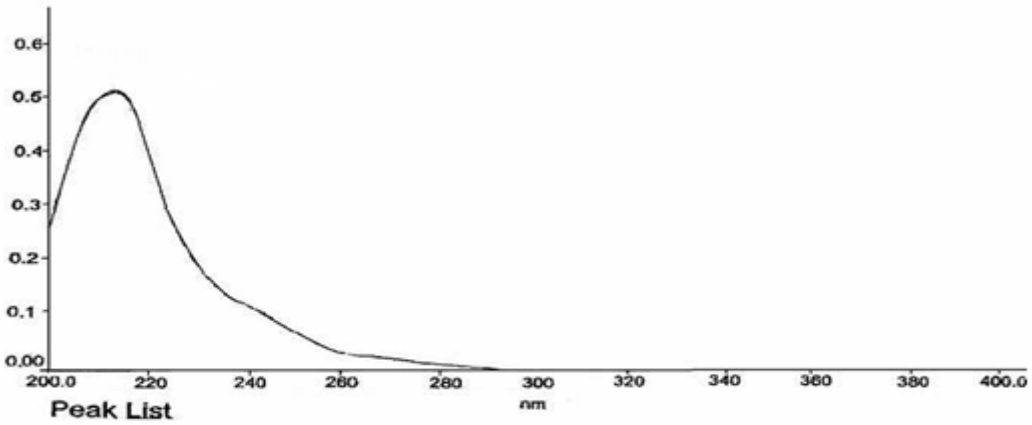


Figure no 1: UV spectrum for Candesartan.

2. HPLC METHOD DEVELOPMENT:

2.1. Optimized Chromatographic Conditions:

Following are the optimized chromatographic condition for RP-HPLC method.

Parameters	Values
Column	Cosmosil C_{18}
Wavelength	230_{nm}
Flow rate	1.2ml/min
Injection volume	20µl
Temperature	Ambient
Runtime	7.93min

2.2. Assay of Candesartan:

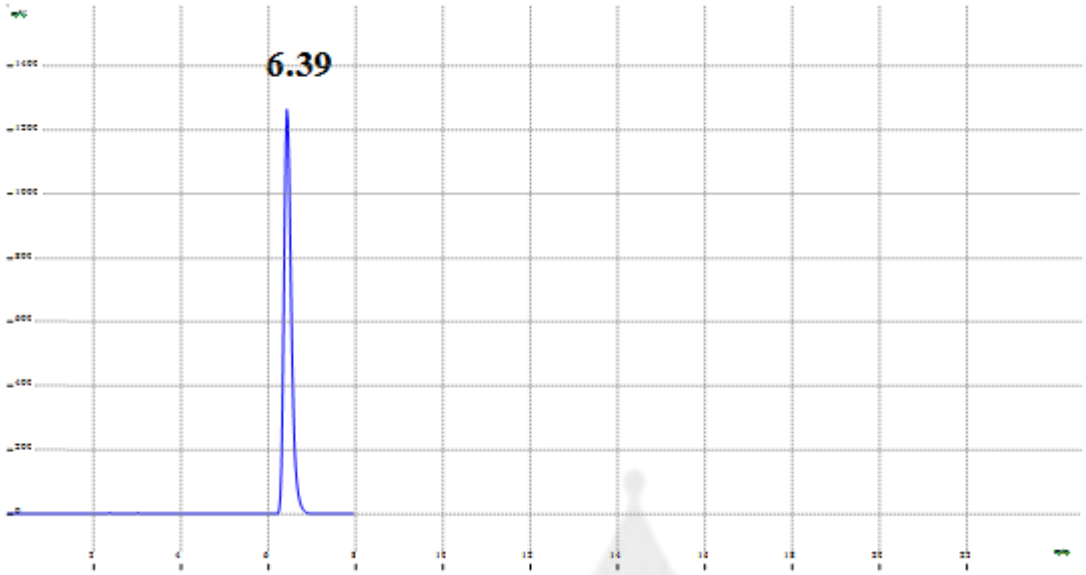


Figure no 2: Chromatograph for standard Candesartan.

Time	Conc.	Area	Resolution	T. Plate	Asymmetry
6.39	10 ug/ml	15043894	00	6592	1.36

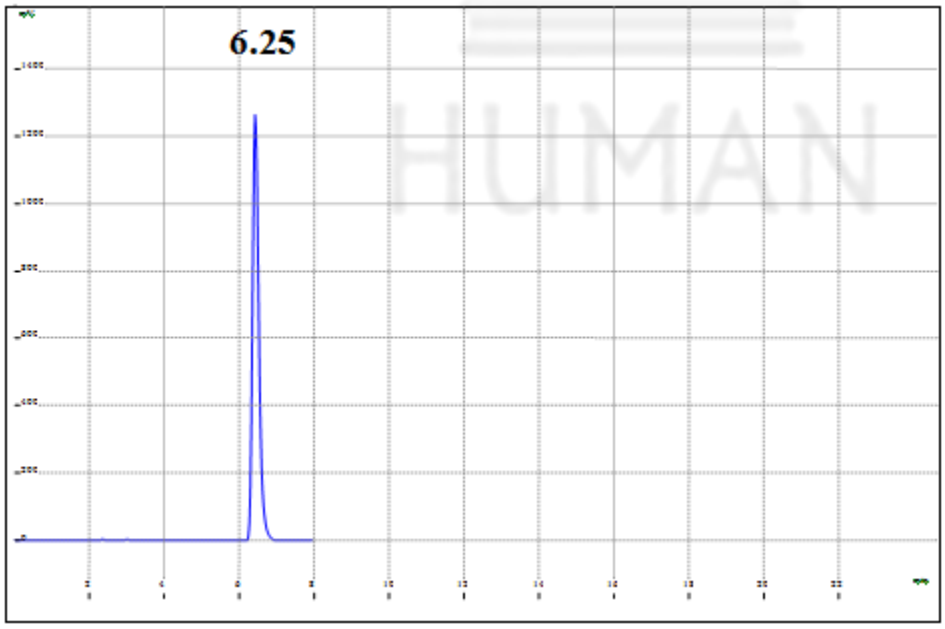


Figure no 3: Chromatograph for Tablet Sample of Candesartan

Time	Conc.	Area	Resolution	T. Plate	Asymmetry
6.25	10 ug/ml	14904195	00	6993	1.34

Data for Assay of Candesartan

Wt. of Std (mg)	Area of standard	Area of sample	Purity of the Std (%)
10	15010535	14905285	100%
	15011056	14903254	
	15023085	14925755	
	15023884	14994451	
	15038656	14913432	
	15043894	14904195	
Mean	15025185	14924395	
SD	13797	35355	
RSD	0.09	0.23	

Percentage Assay obtained for Candesartan is 100 % (Standard-NLT 98.0 and NMT 102.0%). As the result obtained is within the limits, hence this assay method used to perform the validation.

System suitability test for Candesartan

Sr. No.	Area of standard	Retention time (R_t)	USP tailing (T_f)	Theoretical plate count (N)	Resolution (Rs)
1	15010535	6.39	1.25	6532	00
2	15011056	6.36	1.27	6590	00
3	15023085	6.38	1.24	6555	00
4	15023884	6.39	1.25	6584	00
5	15038656	6.37	1.20	6592	00
Average	15031270	6.38	1.24	6570	00
SD	12266				
% RSD	0.08				

System suitability test for Candesartan Tablet.

Sr. No.	Area of sample	Retention time (R_t)	USP tailing (T_f)	Theoretical plate count (N)	Resolution (Rs)
1	14902596	6.24	1.12	6995	00
2	14905585	6.25	1.15	6945	00
3	14924568	6.23	1.10	6912	00
4	14914565	6.24	1.15	6978	00
5	14904195	6.25	1.16	6993	00
Average	14910301	6.24	1.14	6965	00
SD	34255				
% RSD	0.30				

❖ **Acceptance Criteria:**

- RSD should not be more than 2.0 % for five replicate injections of standard
- USP Tailing Factor is not more than 2.0.
- The column efficiency as determined as number of theoretical plates should be more than 8000.

❖ **Conclusion:**

- **%RSD of the was found to be:**

Candesartan: 0.08

Candesartan Tablet: 0.30

- **Number of Theoretical plates was found to be :**

Standard Drug of Candesartan: 6570

Tablet Sample of Candesartan: 6965

- **Tailing factor found was to be:**

Standard drug of Candesartan: 1.24

Tablet sample of Candesartan: 1.14

3. VALIDATION OF THE DEVELOPED RP-HPLC METHOD:

1. Specificity:

Specificity data for proposed HPLC method.

Drug	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Standard Drug of Candesartan	8875756	10	9.91	99.10	1.12	0.40
	8874755	10	9.87	98.70		
Tablet Sample of Candesartan	8765648	10	9.90	99.00	1.15	0.37
	8756657	10	9.88	98.80		

2. Precision:

Intra-Day variability for Std. Drug of Candesartan & Candesartan Tablet.

Drug	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Std Drug of Candesartan.	1	4766000	10	9.65	99.2	0.525	0.101
	2	7128644		9.78	99.1		
	3	9191855		9.85	99.3		
Tablet of Candesartan.	1	4655125	10	9.98	99.8	0.445	0.110
	2	7025567		9.89	99.7		
	3	8775756		9.96	99.8		

Inter-Day variability for Standard Drug of Candesartan and Candesartan Tablet.

Drug	Trial No	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	RSD
Std Drug of Candesartan	1	4766000	10	9.98	99.8	0.387	0.154
	2	7127297		9.88	98.8		
	3	9191885		9.96	99.6		
Tablet of Candesartan	1	4665101	10	10.1	100	0.256	0.220
	2	7028532		9.95	99.5		
	3	9088522		9.97	99.7		

3. Linearity:

Linearity Data for Standard Drug of Candesartan.

Sr. No.	Conc.	Area
1.	**100**	4577414
2.	**120**	6046054
3.	**140**	7062839
4.	**160**	8257643
5.	**180**	9261710

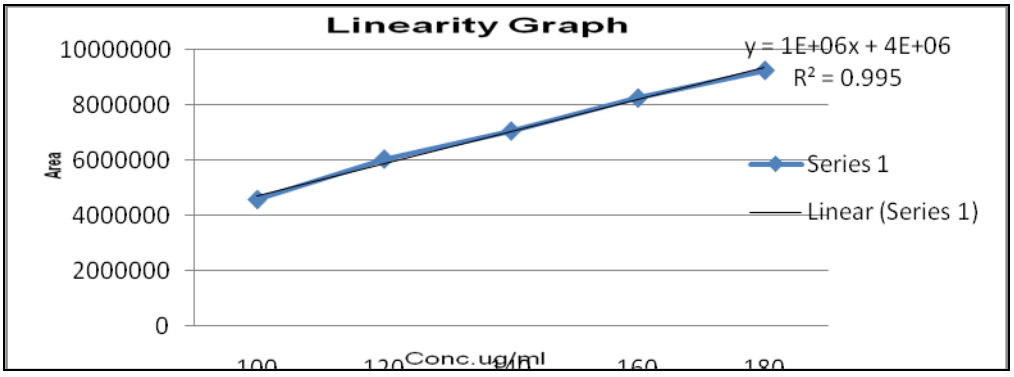


Figure no 4 : Linearity graph for Standard Drug of Candesartan.

Linearity Data for Tablet Sample of Candesartan.

Sr. No.	Conc.	Area
1.	**100**	4465312
2.	**120**	5836037
3.	**140**	6952728
4.	**160**	8154604
5.	**180**	9091821

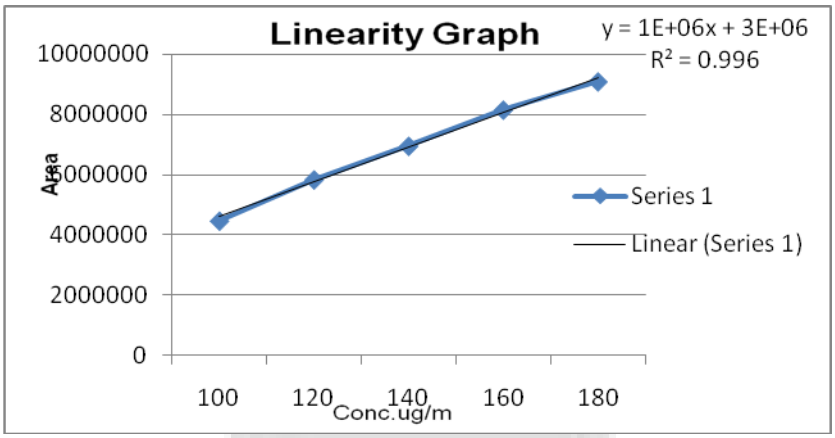


Figure no 5: Linearity graph for Standard Drug of Candesartan.

4. Accuracy (recovery):

Accuracy data of Standard Drug Candesartan and Candesartan Tablet.

Drug	Conc. Of Accuracy ug/ml	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
Std Drug of Candesartan	100	4768984	10	9.85	99.6	1.20	0.65
	140	7078075	10	9.98	98.4		
	180	9196240	10	10.01	100		
Tablet of Candesartan	100	4658803	10	9.95	98.4	0.98	1.01
	140	6945064	10	9.87	99.8		
	180	9085231	10	9.92	99.4		

5. Ruggedness:

Drug	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
Std Drug of Candesartan.	4768982	10	9.86	99.84	0.542	0.061
	7078026	10	9.85	99.84		
Tablet of Candesartan.	4685893	10	9.95	99.50	0.539	0.052
	6968057	10	9.89	99.86		

6. Robustness:

Robustness data of Standard Drug Candesartan and Candesartan Tablet

Drug	Robustness Test ug/ml	Area	Amount added (mg)	Amount recovered (mg)	Percent recovery (%)	SD	% RSD
Standard Drug of Candesartan.	**100**	4781800	10	10	100	0.54	0.85
	120	5841830	10	9.89	99.95		
	140	6661311	10	9.75	99.85		
	160	7593400	10	9.98	99.98		
Tablet sample of Candesartan.	**100**	4680701	10	10.1	100.1	1.25	0.95
	120	5736742	10	9.86	99.86		
	140	6453232	10	9.99	99.90		
	160	7483412	10	9.85	99.80		

7. Limit of Detection and Quantitation:

The limits of detection (LOD) and quantification (LOQ) were determined separately, on the basis of the standard deviation of the y intercept and slope of the calibration plots. The LOD were 0.095 and 0.084µg/ml for Standard Drug of Candesartan and Tablet Sample of Candesartan respectively. For Standard Drug of Candesartan and Tablet Sample of Candesartan the LOQ

were found to be 0.58 and 0.44 µg/ml respectively. At these levels, RSD values were less than 2%, in accordance with ICH guidelines.

CONCLUSION

This developed RP-HPLC method for estimation of Candesartan is accurate, precise, robust, specific and stability-indicating. The method has been found to be better than previously reported method, because of its less retention time, use of an economical and readily available mobile phase, UV detection and better resolution of peaks. The run time is relatively short, which will enable rapid quantification of many samples in routine and quality-control analysis of various formulations containing Candesartan. All these factors make this method suitable for quantification of Candesartan in bulk drugs and in pharmaceutical dosage forms without any interference. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and specific.

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