ISSN: 2321-5038
IJRPLS, 2013, 1(2): 131-137
Research Article

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND LIFE SCIENCES
www.pharmaresearchlibrary.com/ijrpls

Analytical Method Development and Validation for the Simultaneous Estimation of Candesartan and Hydrochlorothaizide by RP-HPLC Method Pharmaceutical Dosage Form

Devanapelly Shashikantha Rao*
Lecturer, Department of Chemistry, C.K.M. Arts and Science College, Warangal- 506006, Andhra Pradesh, India
*E-mail: shashikanth.dev@gmail.com

Abstract
A new method was established for simultaneous estimation of Candesartan cilexitil and Hydrochlorothiazide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Candesartan cilexitil and Hydrochlorothaizide by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid),detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.170 mins and 7.025 mins. The % purity of Candesartan cilexitil and Hydrochlorothaizide was found to be 99.1% and 98.2% respectively. The system suitability parameters for Candesartan cilexitil and Hydrochlorothaizide such as theoretical plates and tailing factor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Candesartan cilexitil and Hydrochlorothaizide was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively.
Key words: Candesartan Cilexitil, Hydrochlorothiazide, HPLC

1. Introduction

Analytical methods
Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness[1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available[2].

Description of the Various Analytical Methods
Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture[4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

Chromatography:
Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The

name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate[7]. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC [9].

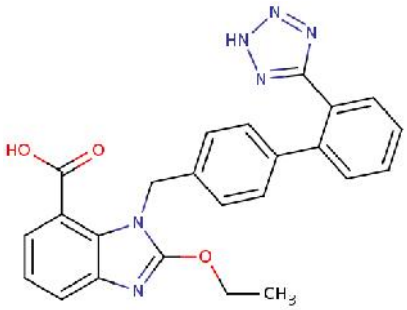

Figure 1: Candesartan cilexitil

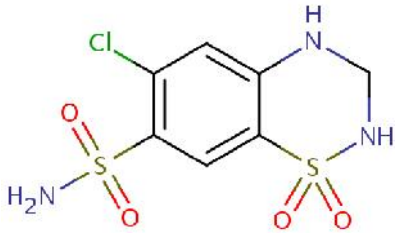

Figure 2: Hydrochlorothiazide

2. Materials and Method

Apparatus

Waters HPLC-auto sampler –UV detector Separation module2695, UV.detector2487 Empower-software version-2. U.V double beam spectrometer, Digital weighing balance, P^H meter.

Reagents and Materials

The solvents used were Potassium dihydrogen phosphate, K_2HPO_4, Methanol, Acetonitril and Water [10].

Selection of detection wavelength:

10 mg of Candesartan cilexitil and Hydrochlorothaizide was dissolved in mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Candesartan cilexitil and Hydrochlorothaizide. The isobestic point was taken as detection wavelength [11].

Selection of mobile phase

- pH 3 phosphate buffer : Methanol (70 : 30% v/v)
- Buffer pH should be between 2 to 8.
- Below 2: siloxane linkages are cleaved.
  Above 8: dissolution of silica.
- pH selected: 3 ±0.05
- pH controls the elution properties by controlling the ionization characteristics.
- Reasons: To decrease the retention and improve separation. Good Response, Area, Tailing factor, Resolution [12].

Optimization Chromatographic trials for Simultaneous Estimation of Candesartan Cilexitil and Hydrochlorothiazide by RP- HPLC.

Optimization chromatographic conditions

Column	:	Zodiac sil C18 column (4.6×150mm)5µ
Mobile phase ratio	:	Methanol: pH 3 phosphate buffer (70: 30 % v/v)
Detection wavelength	:	240 nm
Flow rate	:	1.0ml/min
Injection volume	:	20µl
Column temperature	:	Ambient
Auto sampler temperature	:	Ambient
Run time	:	10min
Retention time	:	2.170 and 7.280 min

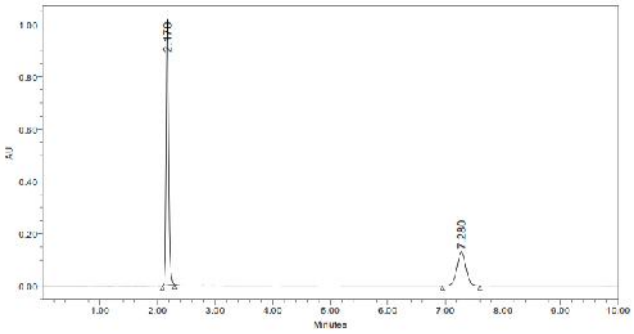

Figure 3: Optimization Chromatogram

Observation: The separation was good, peak shape was good, so we conclude that there is no required for reduce the retention times of peaks, so it is taken as final method.

Procedure

Standard solution preparation

Accurately weigh and transfer 12.5 mg & 8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent [13].

Stock solution: Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents [14].

Procedure: 10µL of the blank, standard and sample were injected into the chromatographic system and areas for the Candesartan cilexitil and Hydrochlorothiazide the peaks were used for calculating the % assay by using the formula [15].

Sample solution preparation:

Accurately weigh and transfer 59.8 mg of Hydrochloro thiozide and Candesartan Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [16]. (Stock solution). Further pipette 0.6ml of Hydrochlorothiazide& Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents[17].

3. Results and Discussion

Method Validation Parameters

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by injecting blank.

2. Linearity

Preparation of stock solution:

Accurately weigh and transfer 12.5 mg & 8mg of Hydrochloro thiazide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [18].

Preparation of Level – I (25ppmof Hydrochloro thiazide&16ppmof Candesartan):

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (50ppmof Hydrochloro thiazide&32ppmof Candesartan):

0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (75ppmof Hydrochloro thiazide&48ppmof Candesartan):

0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (100ppmof Hydrochloro thiazide&64ppmof Candesartan):

0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (125ppmof Hydrochloro thiazide&80ppmof Candesartan)

1.0 of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Each level was injected into the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and the correlation coefficient was calculated [19].

Acceptance criteria: Correlation coefficient should be not less than 0.999.

3. Range: Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 16µg/ml-80µg/ml and 25µg/ml to 125µg/ml of Candesartan cilexitil and Hydrochlorothaizide respectively [20].

4. Accuracy

Preparation of standard stock solution

Accurately weigh and transfer 12.5 mg &8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.6ml of Hydrochloro thiozide&Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents[21].

Preparation of sample solutions

For preparation of 50% solution (with respect to target assay concentration)

Accurately weigh and transfer 7mg of Hydrochloro thiozide and 4.25mg of Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate dissolve it completely and make volume up to the mark with the same solvent [17].

Stock Solution:

Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

For preparation of 100% solution (with respect to target assay concentration)

Accurately weigh and transfer 13.1mg of Hydrochloro thiozide and 8.25mg of Candesartan working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [21].

Stock solution: Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% solution (with respect to target assay concentration)

Accurately weigh and transfer18.5mg of Hydrochloro thiozide and 12.2mg of Candesartan working standards into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent [19].

Stock solution: Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluents.

Procedure

The standard solutions of accuracy 50%, 100% and 150%were injected into chromatographic system. Calculate the amount found and amount added for Candesartan cilexitil and Hydrochlorothaizide and calculate the individual % recovery and mean % recovery values [20].

Acceptance criteria: The % recovery for each level should be between 98.0 to 102.0%

5. Precision

Repeatability

Preparation of stock solution:

Accurately weigh and transfer 12.5 mg &8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent.

Stock solution: Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria: The % RSD for the area of five standard injections results should not be more than 2.

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different days by using different make column of same dimensions.

Preparation of stock solution

Accurately weigh and transfer 12.5 mg &8 mg of Hydrochloro thiozide and Candesartan working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonic ate to dissolve it completely and make volume up to the mark with the same solvent[21].

Stock solution

Further pipette 0.6ml of Hydrochloro thiozide & Candesartan the above stock solution into a10ml volumetric flask and dilute up to the mark with diluent

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria: The % RSD for the area of five sample injections results should not be more than 2%.

Validation of the Method

1. Specificity: The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank.

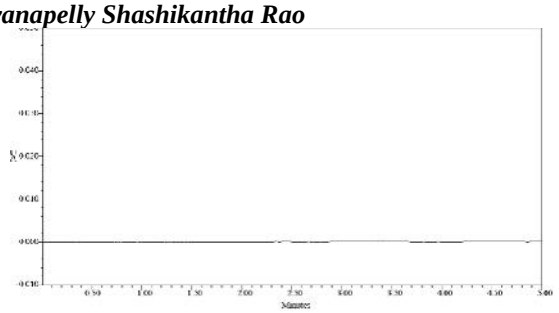

Figure 4: Chromatogram of Blank

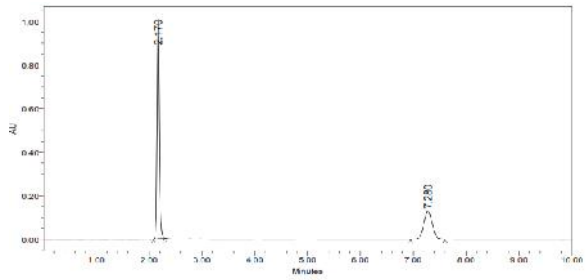

Figure 5: Chromatogram of Sample

2. Linearity: The linearity study was performed for the concentration of 16 ppm to 80ppm for Candesartan cilexitil and 25ppm to 125ppm for Hydrochlorothiazide and chromatograms are shown below.

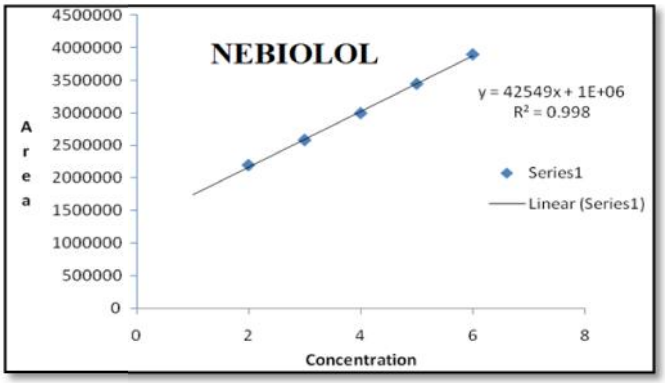

Figure 6: Calibration graph of Candesartan cilexitil

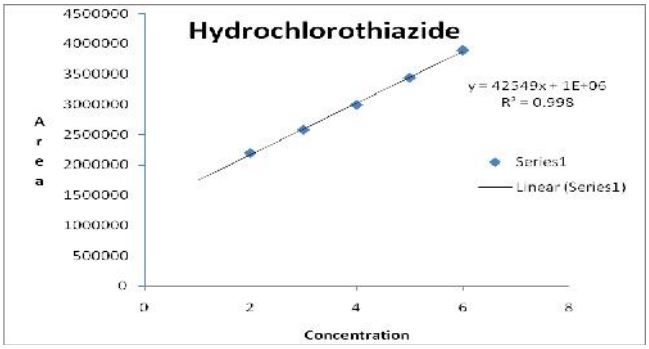

Figure 7: Calibration graph of Hydrochlorothiazide

Table 1: Linearity Results for Candesartan cilexitil

S.No	Linearity Level	Concentration	Area
1	I	16ppm	1027461
2	II	32ppm	1233566
3	III	48ppm	1437030
4	IV	64ppm	1644336
5	V	80ppm	1880590
Correlation Coefficient			**0.999**

Table 2: Linearity Results for Hydrochlorothaizide

S.No	Linearity Level	Concentration	Area
1	I	25ppm	2201022
2	II	50ppm	2585033
3	III	75ppm	2996553
4	IV	100ppm	3446224
5	V	125ppm	3897922
Correlation Coefficient			0.999

Recovery studies: Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 3: Accuracy results for Candesartan cilexitil

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	765624	4.25	4.30	101.2%	101.4%
100%	1508055	8.25	8.48	101.5%	
150%	2204983	12.2	12.39	101.6%	

Table 4: Accuracy (recovery) data for Hydrochlorothiazide

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1726242	7.05	7.1	101.9%	101.7%
100%	3187170	13.1	13.2	101.3%	
150%	4521881	18.5	18.8	101.8%	

Table 5: Showing results for intermediate precision of Candesartan cilexitil

Injection	Area
Injection-1	1419430
Injection-2	1437396
Injection-3	1461998
Injection-4	1484335
Injection-5	1486671
Injection-6	1488969
Average	1463133.2
Standard Deviation	29136.557

Table 6: Showing results for intermediate precision of Hydrochlorothaizide

Injection	Area
Injection-1	3098177
Injection-2	3075703
Injection-3	3135114
Injection-4	3173644
Injection-5	3179888
Injection-6	3184696
Average	3141203.7
Standard Deviation	46085.033
%RSD	1.46

4. Conclusion

A new method was established for simultaneous estimation of Candesartan cilexitil and Hydrochlorothaizide by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Candesartan cilexitil and Hydrochlorothaizide by using Zodiac sil C18 column (4.6×150mm)5µ, flow rate was 1ml/min, mobile phase ratio was (70:30 v/v) methanol: phosphate buffer ($KH_2PO_4$and K_2HPO_4) phosphate pH 3 (pH was adjusted with orthophosphoricacid), detection wavelength was 240nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.170 mins and 7.025 mins. The % purity of Candesartan cilexitil and Hydrochlorothaizide was found to be 99.1% and 98.2% respectively. The system suitability parameters for Candesartan cilexitil and Hydrochlorothaizide such as theoretical plates and tailing factor were found to be 12294, 1.27 and 10491 and 1.03, the resolution was found to be 8.67. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Candesartan cilexitil and Hydrochlorothaizide was found in concentration range of 16µg-80µg and 25µg-125µg and correlation coefficient (r^2) was found to be 0.999 and 0.998, % recovery was found to be 101.7% and 102.0%, %RSD for repeatability was 0.8and 0.5, % RSD for intermediate precision was 1.99 and 1.82 respectively. The precision study was precision, robustness and repeatabilty.LOD value was 2.17 and 0.0372 and LOQ value was 6.60 and 0.1125 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Candesartan cilexitil and Hydrochlorothaizide in API and Pharmaceutical dosage form.

5. References

1. Willard HH, Merrit LL, Dean JA, Settle FA. Instrumental methods of analysis, CBS Publishers and Distributors, New Delhi, 6th edition, 1986, 1-15.
2. Douglas A. Skoog, F. James Holler, Timothy A. Nieman. Principles of instrumental analysis, Saunders Golden Sun burst Series, Philadelphia, 2nd edition, 1980, 725-760.
3. David G.Watson. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists, Harcourt Publishers Limited, 2nd Edition, 1999, 221-232, 267-311.
4. Snyder LR, Kirkland JJ, Joseph LG. Practical HPLC Method Development, Wiley Inter Science, New York, 2nd Edition, 1997, 1-56, 234-289,685-712.
5. Beckett A.H, J.B. Stenlake. Practical Pharmaceutical Chemistry, 4th edition. C.B.S. Publications, Pg. No.53-62.
6. Remingtonn's The Science and Practise of Pharmacy,20th Edition, 2000.
7. Connors KA. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc, New Delhi, 3rd Edition, 1994, 373-421.
8. Rashmin.B.Patel, Mrunali R. Patel, An Introduction to Analytical method development for pharmaceutical formulations, Pharmainfo.net 2008; 17:19
9. Sharma B.k Instrumental methods of chemical analysis. 19 ed: Goel Publishing House, 2003.
10. Galen Wood Ewing, Instrumental methods of chemical analysis, 340-345.
11. United States of Pharmacopeia, USP30-NF25, The official compendia of standards, official May 1, 2007.
12. ICH topic Q2B, validation of analytical procedure & methodology, The European agency for evaluation of medicinal products, human medicines evaluation unit 1996.
13. ICH: Q2A, Text on validation of analytical procedure (October 1994).
14. .Namala Durga Atchuta Kumar et al, A validated ultra high-pressure liquid chromatography method for separation of candesartan cilexetil impurities and its degradents in drug product. Pharm Methods. 2012 Jan-Jun; 3(1): 31–39.
15. Veeranjaneyulu D et al, Stability Indicating RP-HPLC Method For The Simultaneous Determination of Candesartan Cilexetil And Hydrochlorothiazide In Bulk And Dosage Forms. Indian Journal of Research in Pharmacy and Biotechnology ISSN: 2321-5674(Print) ISSN: 2320 – 3471
16. R Revathi et al, Development and validation of a dissolution test for Candesartan cilexetil in tablet forms using reverse phase – High performance liquid chromatography.J Pharm Educ Res Vol. 2, Issue No. 2, December 2011.
17. M. Mathrusri Annapurna et al, Liquid Chromatographic Method For The Simultaneous Quantitative Determination Of Candesartan Cilexetil And Hydrochlorthiazide In Pharmaceutical Dosage Forms.Journal of Drug Delivery & Therapeutics; 2012, 2(2): 213-215.
18. SURAJ SAHOO et al, Hplc Method Development For Simultaneous Estimation Of Hydrochlorothiazide And Perindropril In Tablet Dosage Form.Asian Journal of Pharmaceutical and Clinical Research Vol 5, Suppl 2, 2012.
19. D Vijaya Bharathi*, Kishore Kumar Hotha, Pankaj K Chatki et al, LC–MS/MS method for simultaneous estimation of candesartan and hydrochlorothiazide in human plasma and its use in clinical pharmacokinetics. Reaserch article,Vol. 4, No. 10, Pages 1195-1204.
20. Manisha P Puranik, Sailesh J Wadher, Ashish L Kosarkar and Pramod G Yeole et al, Method Development and Validation of Candesartan cilexetil by RP-HPLC. International Journal of Research in Pharmaceutical and Biomedical Sciences. Vol. 3 (3) Jul – Sep2012.
21. Pradhan KK, Mishra US, Pattnaik S, Panda CK, Sahu KC et al, Development and Validation of a Stability-indicating UV Spectroscopic Method for Candesartan in Bulk and Formulations. Indian J Pharm Sci. 2011 Nov; 73(6):693-6.