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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF AZILSARTAN MEDOXOMIL API AND ITS APPLICATION TO FORCED DEGRADATION STUDIES

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Abstract:

A simple, specific and accurate stability indicating reverse phase liquid chromatographic method was developed for the estimation of Azilsartan Medoxil in its bulk form. Azilsartan medoxil is an angiotensin-II receptor antagonist used in the treatment of hypertension. Chromatography was performed using C18 column Qualisil Gold (250 X 4.6 mm, 5µm) with mobile phase consisting 0.2% trifluroacetic acid in acetonitrile and 0.2% trifluroacetic acid in MilliQ water in the ratio of 62:38.The pH was adjusted to 3 with orthophosphoric acid. The detection was carried out at 248nm and retention time (RT) of Azilsartan Medoxil was found to be 7.353min.The developed method was statistically validated for linearity(20-120 µg/ml) and the results of precision, accuracy, specificity, LOD(0.0186µg/ml), and LOQ(0.0613 µg/ml) were well within limits. Azilsartan Medoxil was subjected to stress conditions including acidic, alkaline, oxidative, photolysis and thermal degradation and the results showed that it was highly sensitive to alkaline conditions followed by liable to photolytic, oxidative, thermal, acidic and neutral stress conditions. The degraded products were well resolved from the analyte peak with significant difference in their RT values. The method was validated as per ICH guidelines.

Keywords: *Azilsartan medoxil, Reverse phase, ICH, Chromatography.*

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INRODUCTION:

Azilsartan medoxil chemically known as (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 2-ethoxy-1- {[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]methyl}-1*H*-benzimidazole-7-carboxylate,is a white powder, practically insoluble in water ,freely soluble in methanol. Azilsartan medoxil is an antihypertensive drug used in treatment of hypertension. it is a selective AT_1subtype angiotensin-II receptor antagonist.Chromatographic methods are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products and compounds in biological fluids. The components monitored include chiral or achiral drug, process impurities, residual solvents, excipients such as preservatives, degradation products, extractable and leachable from container and closure or manufacturing process, pesticide in drug product from plant origin and metabolites.The objective of the test method is to generate reliable and accurate data regardless of whether it is for acceptance, release, stability or pharmacokinetic study. Data are generated for the qualitative and quantitative testing during development and post approval of the drug products. The testing includes the acceptance of raw materials, release of the drug substances and products, in process testing for quality assurance and establishment of the expiration- dating period.

The specific aim of the research was:To develop a RP-HPLC method for the estimation of Azilsartan Medoxomil API.Validate the proposed methods in accordance with ICH guidelines for the intended analytical application.Forced degradation studies of Azilsartan Medoxomil API.

MATERIALS AND METHOD:

Azilsartan Medoxomil(Aurobindo Pharma Limited,Hyderabad (India)), HPLC solvents: Acetonitrile, water (Merck, Mumbai (India), Orthophosphoric acid(Qualigens, Mumbai), Triethyl amine(Qualigens, Mumbai), pH meter(Systronics, Digital pH meter 802).

System validation:

Table No.1: System validation

S.No	HPLC module	Test	Acceptance criteria
1	Pump	Flow accuracy Flow precision	< ±5% RSD < ±0.5%
2	Detector	Wavelength accuracy	λ_{max} ± 3 nm

Validation of pump

a) Flow accuracy: It was determined by collecting the mobile phase at the outlet into a 10ml volumetric flask and time was measured.

b) Flow Precision: It can be determined by injecting six 10µl injections of ethyl paraben (20 µg/ml), determine retention time RSD.

Validation of detector

Wavelength accuracy: Anthracene solution (1mg/ml) was prepared in methanol and injected.

Chromatographic conditions

The mobile phase consisted of acetonitrile, water (0.2% triethyl amine buffer) in the ratio 62:38 (adjusted to pH 3 with orthophosphoric acid) contents of the mobile phase were filtered before use through a 0.45µ membrane filter and degassed for 15 minutes. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min and the injection volume was 20 µl. The eluents were monitored at 248 nm.

Calibration of standards

Different volumes of stock solutions were accurately transferred in to 10 ml volumetric flasks and diluted to mark to yield concentration range of 20-120 µg/ml for Azilsartan Medoxomil. Six solutions were prepared and the final volume was made upto the mark with mobile phase. The calibration curve was obtained by plotting the peak area against the concentration of drug.

Method validation of Azilsartan Medoxomil

Method of validation was performed in terms of specificity and selectivity, linearity, LOD, LOQ, accuracy, precision, and robustness.

RESULTS:

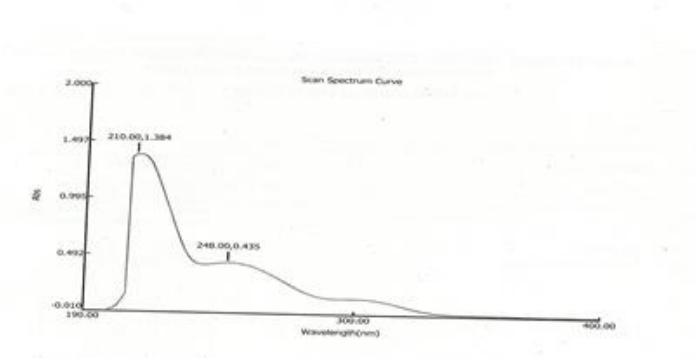

Fig1: Spectra of Standard APIs:

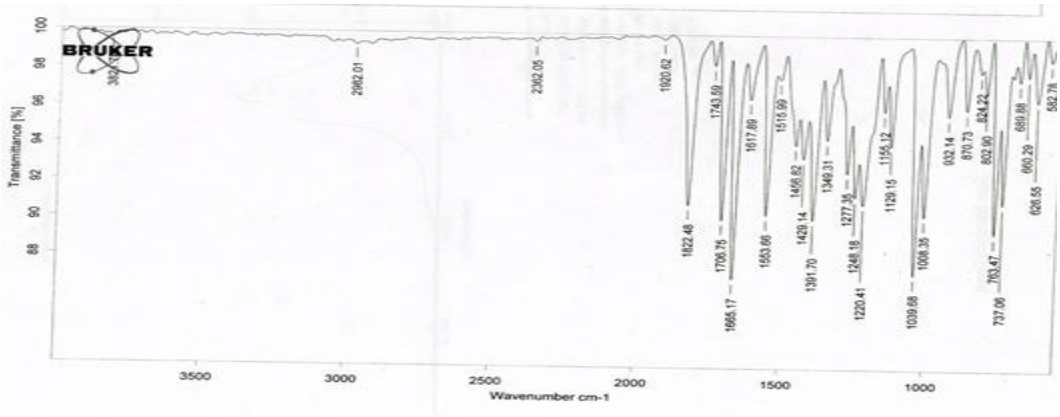

Fig 2: IR spectrum of Azilsartan Medoxomil

Validation of Azilsartan Medoxomil

Specificity and Selectivity

Specificity is the ability of a method to discriminate between the intended analyte(s) and other components in the sample. Selectivity of the HPLC method is demonstrated by the separation of the analytes from other potential components such as impurities, degradants, or excipients.

Determination**:** Volume of 20 µl of working placebo sample solution was injected into the chromatograph and the chromatogram was recorded and presented below.

Observation: No peaks were found at retention time of 7.380 minutes and the drug was clearly separated from its degradants.

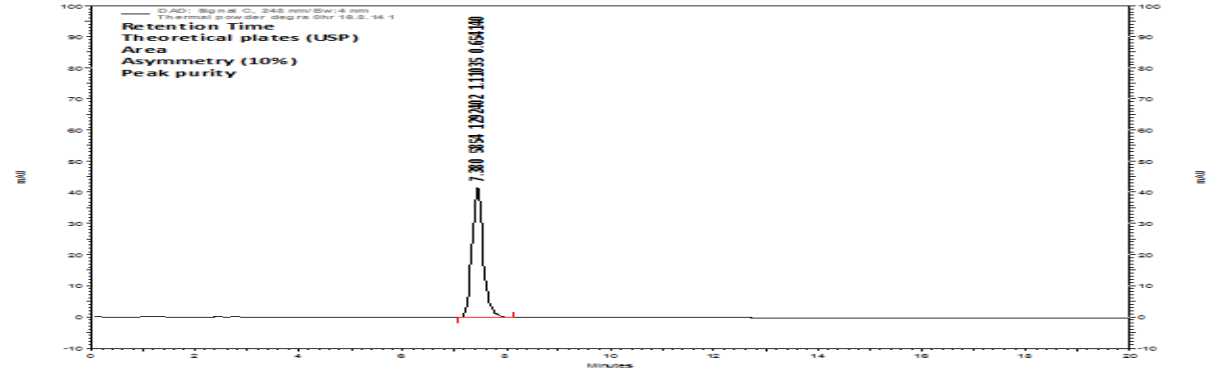

Fig 3: Chromatogram of Azilsartan Medoxomil (control) – Specificity

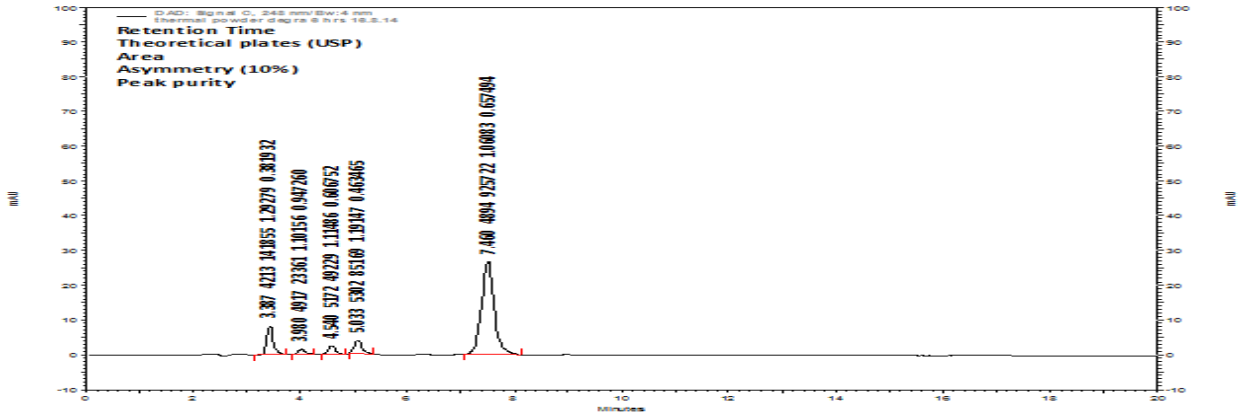

Fig 4: Chromatogram of degraded sample – Selectivity

Linearity

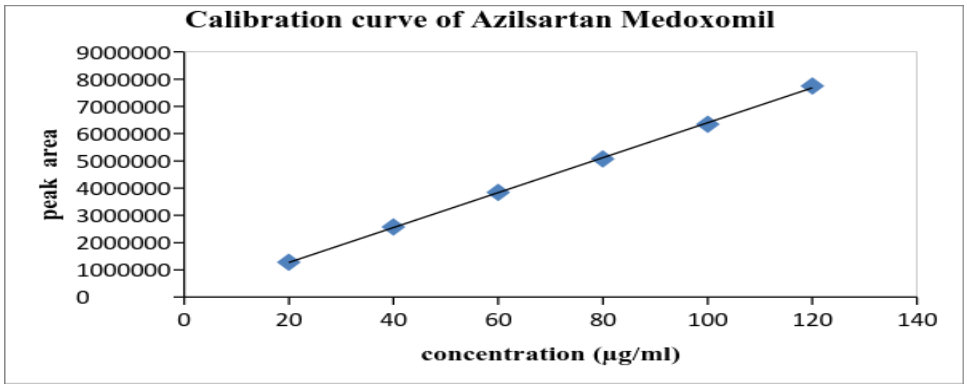

Fig 5: Calibration curve of Azilsartan Medoxomil

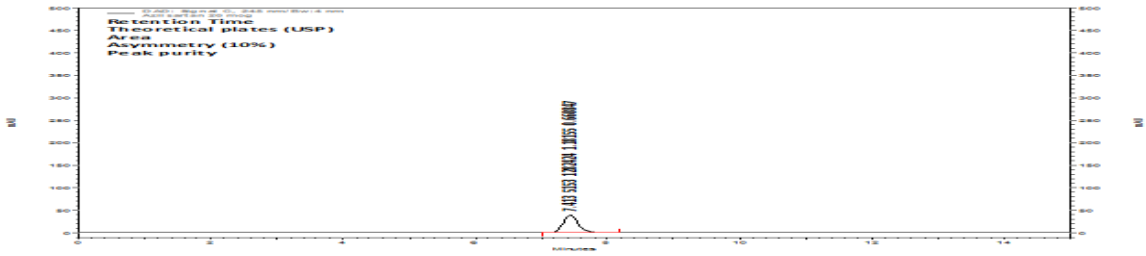

Fig 6: Chromatogram for linearity of 20 µg/ml

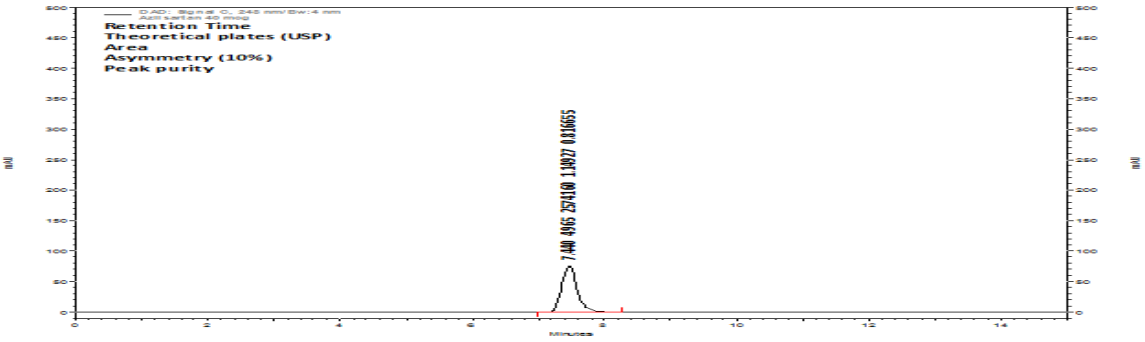

Fig 7: Chromatogram for linearity of 40 µg/ml

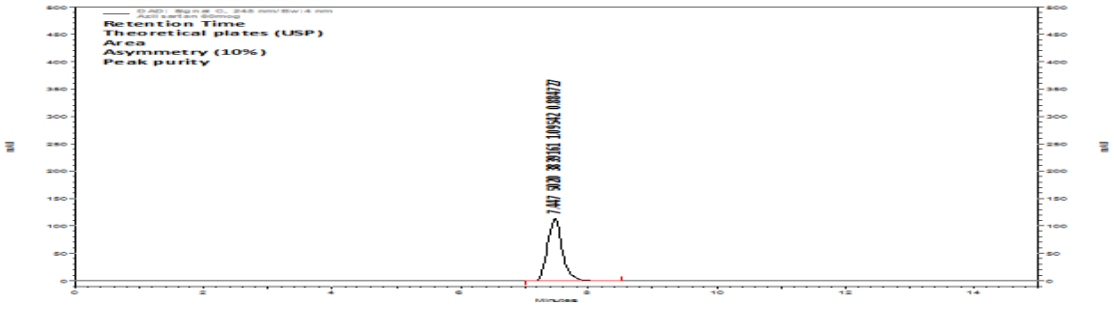

Fig 8: Chromatogram for linearity of 60 µg/ml

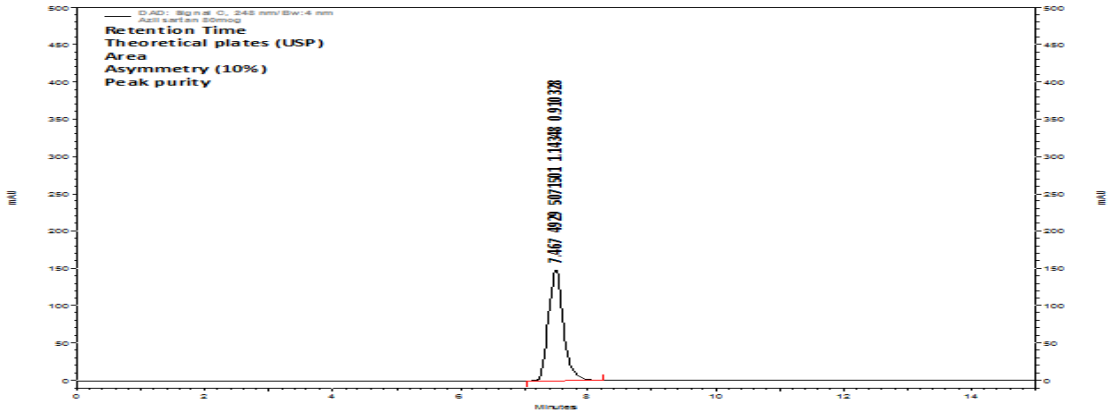

Fig 9: Chromatogram for linearity of 60 µg/ml

Precision

System Precision (Repeatability)

Table 2: System precision of Azilsartan Medoxomil

Injection No	Concentration (µg/ml)	Peak area at 248 nm	R_t (min)
1	50	3183070	7.413
2	50	3178312	7.433
3	50	3185732	7.440
4	50	3182152	7.427
5	50	3175213	7.447
6	50	3180541	7.407
Mean ± SD		3180836.67 ± 3708.62	7.4278 ± 0.015
% RSD		0.12	0.20

Table 3: Method precision of Azilsartan Medoxomil

Injection No	Concentration (µg/ml)	Peak area at 248 nm	R_t (min)
1	50	3214309	7.467
2	50	3192612	7.423
3	50	3198753	7.413
4	50	3211532	7.353
5	50	3209210	7.441
6	50	3216821	7.347
7	50	3195423	7.443
8	50	3205157	7.440
Mean ± SD		3205477.13 ± 9017.40	7.4159 ± 0.044
% RSD		0.28	0.59

Accuracy

Table 4: Recovery studies of Azilsartan Medoxomil

S.No	Pre-analysed sample concentration (µg/ml)	Recovery level	Amount added (µg/ml)	Amount of drug found (µg/ml), (n=3) mean ± SD	% recovery	% RSD
1	50	80%	40	40.096 ± 0.085	100.24	0.21
		100%	50	49.960 ± 0.127	99.92	0.25
		120%	60	60.175 ± 0.248	100.29	0.41

Assay

Table 5: Assay of Azilsartan Medoxomil

S.No	Active pharmaceutical ingredient (API)	Concentration (µg/ml)	Amount found (µg), (n=3) Mean ± SD	% Assay	% RSD
1	Azilsartan Medoxomil	50	49.822 ± 0.141	99.64	0.28

Limit of detection (LOD) and Limit of Quantitation (LOQ)

Table 6: LOD and LOQ report of Azilsartan Medoxomil

S. No	Drug	LOD	LOQ
1	Azilsartan Medoxomil	0.0186 µg/ml	0.0613 µg/ml

Robustness

Table No 7: Robustness studies of Azilsartan Medoxomil

Parameter	Conditions	R_t (min)	Area (n=3)	% Assay	Remarks
Optimized	ACN: water (0.2% TEA, pH 3) in ratio of 62:38, 1ml/min, λ_{max} : 248nm	7.353	1259188 ± 3042	100	-------
Flow rate	0.9 ml/min	8.167	1401371 ± 8956	111.29	Not Robust
	1.1 ml/min	6.693	1145763 ± 7913	90.99	Not Robust
Mobile phase	ACN: water (60:40)	8.280	1254958 ± 8429	99.66	Robust
	ACN: water (64:36)	6.693	1269673 ± 5575	100.83	Robust
pH	2.9	7.347	1275086 ± 6152	101.26	Robust
	3.1	7.447	1278649 ± 7325	101.55	Robust
Wavelength	246 nm	7.353	1246638 ± 5548	99.00	Robust
	250 nm	7.353	1258092 ± 4150	99.91	Robust

System suitability

Table 7: System suitability parameters of Azilsartan Medoxomil

S.No	Parameter	Values obtained	Acceptance criteria
1	Retention time	7.353	----
2	Theoretical plates	5190	> 2000
3	Peak Asymmetry	1.05	≤ 1.5

CONCLUSION:

In present study the Azilsartan Medoxomil (Selective AT_1 subtype angiotensin II receptor antagonist) the essential therapeutic agent in treatment of hypertension. Among the analytical techniques available in the estimation and quantification, HPLC method is an emerging technique reliable in vast areas of research that incited the author to undertake method development and validation as per ICH guidelines for the same.

In the present work forced degradation HPLC method has been developed for the estimation of Azilsartan Medoxomil API. Forced degradation HPLC method was developed with mobile phase system of acetonitrile: water (0.2% TEA, pH 3 adjusted with OPA) in the ratio of 62: 38 v/v. The flow rate of 1 ml/min was used on C_{18} column (250 x 4.6 mm, 5 µm particle size). The retention time of Azilsartan Medoxomil was observed at 7.353 min.

REFERENCES:

1.L.R. Snyder and J.J. Kirkland, Introduction to modern liquid chromatography, 2nd edition, John wiley & sons, New York, 1979.

2.L.R. Snyder and J.J. Kirkland, and J.L. Glajch, Practical HPLC method development, 2nd edition, Wiley- inter science, New York, 1997.

3.Michael W. Dong, Modern HPLC for practicing scientists, John wiley & sons, New Jersey (2006) 193-196.

4.Hokanson GC. A life cycle approach to the validation of analytical methods during pharmaceutical product development. Part I: The initial validation process. Pharma Tech (1994) 118-30.

5.Hokanson GC. A life cycle approach to the validation of analytical methods during pharmaceutical product development. Part II: Changes and the need for additional validation. Pharma Tech (1994) 92-100.

6.Green JM. A practical guide to analytical method validation, Anal Chem (1996) 305A- 309A.

7.FDA, guidance for industry: analytical procedures and method validation (draft guidance), Food and Drug Administration, Rockville, MD, 2000.

8.General chapter 1225, Validation of compendial methods, United States Pharmacopoeia 30, National Formulary 25, Rockville, MD., USA, The United States Pharmacopoeial Convention, Inc., (2007).

8.ICH, Validation of analytical procedures: Text and methodology. International Conference on Harmonisation, IFPMA, Geneva, 1996.

9.http://www.labcompliance.com/tutorialmethods/default.aspx.

10.ICH, Stability testing of new drug substances and products. International Conference on Harmonisation, IFPMA, Geneva, 1993.

11.J.T. Carstensen, C.T. Rhodes (Eds), drug stability: Principles and practices, Marcel Deckker, New York, 2000, P.339.

12.http://www. Cmcissues.com/stability/stress_stability.htm.

13.http://www.pharmainfo.net/stability articles/available guidance and best practices conducting forced degradation studies.

14.ICH, Photostability testing of new drug substances and products. International Conference on Harmonisation, IFPMA, Geneva, 1993.

15.FDA, guidelines for industry: drug stability guidelines. Food and Drug Administration, Rockville, MD, 2000.

16.http://www.fda.gov/downloads/AnimalVeterinary/guidanceComplianceEnforcement/GuidanceforIndustry.

17.Clarke's analysis of drugs and poisons, 3rd edition, 2005.

18.http://www.drugbank.ca/drugs/DB08822

19.http://www.en.wikipedia.org/wiki/azilsartan

20.http://www.medindia.net/doctors/drug_information/azilsartan.htm

21.Paras P. Vekariya and Hitendra S. Joshi, Development and validation of RP-HPLC method for Azilsartan Medoxomil potassium quantitation in human plasma by solid phase extraction procedure, Hindawi Publishing Corporation, ISRN

Spectroscopy, Volume 2013, Article ID 572170, 1-6.
22.Madhu Babu Kasimala, Bikshal Babu Kasimala, Reverse phase-hplc method development and validation for the simultaneous estimation of Azilsartan Medoxomil and Chlortalidone in pharmaceutical dosage forms, Journal of Atoms and Molecules, 2(1); 2012 / 117–126.
23.S. Naazneen, A. Sridevi, Stability-indicating RP-HPLC method for the simultaneous estimation of Azilsartan Medoxomil and Chlorthalidone in solid dosage forms, International Journal of Pharmacy and Pharmaceutical Sciences, 2014, Vol 6, Issue 6, 236-243.
24.Raja Gorla, CH. Nagaraju, B. Sreenivasulu, N. Sreenivas1 and Raghu Babu Korupolu, New simple UV Spectrophotometric Method for determination of Azilsartan Medoxomil in bulk and pharmaceutical dosage forms, International Journal of Research in Pharmaceutical and Biomedical Sciences, Oct – Dec 2013, Vol. 4 (4), 1133-1137.