International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 2, 2016

Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF AZILSARTAN MEDOXIMIL AND CHLORTHALIDONE IN BULK FORM AND FORMULATION USING QUALITY BY DESIGN

SANDEEP KUMAR SOHNI[1], ROBIN KUMAR[2], MYMOONA AKHTAR[1], CHANDA RANJAN[1], GITA CHAWLA[1*]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India, [2]Reference Standard Division, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare, Govt. of India, Sector-23, Rajnagar, Ghaziabad 201002, India
Email: gchawla@jamiahamdard.ac.in

Received: 13 Aug 2015 Revised and Accepted: 30 Dec 2015

ABSTRACT

Objective: Development of an accurate, precise, robust, sensitive, economical and rapid isocratic reversed-phase high-performance liquid chromatography (RP-HPLC) method complying quality by design (QbD) trends for simultaneous estimation of azilsartan medoximil and chlorthalidone in bulk and formulation form and validation of the method as per ICH guidelines.

Methods: The simultaneous estimation of the drugs-azilsartan and chlorthalidone was performed using C8 column having dimensions 150×4.6 mm×5 µm, injection volume 10 µl, flow rate 0.8 ml/min., runtime 10 min., column temperature 20 °C, sampler temperature 5 °C and ultraviolet detection using a photodiode array detector at 220 nm as constant. The optimized method was validated as per ICH guidelines.

Results: The retention times for chlorthalidone and azilsartan medoxomil were 2.4 min. and 5.1 min. respectively with resolution 17. The method was validated as per the ICH guidelines. The linearity of chlortalidone and azilsartan medoxomil was in the range of 6.3 to 15 µg/ml and 20 to 48 µg/ml respectively. The potency of the formulation was found to be 108.12 % and 98.20 % respectively, which are within acceptable limits as per IP.

Conclusion: Method validation results have proven the method to be selective, precise, accurate, and robust, as well as stability indicating. The C8 column used for analysis gave encouraging results with better resolution and less retention time. This method can be successfully applied for the routine analysis involving the determination of content uniformity and dissolution profiling as well as stability study by the industry.

Keywords: RP-HPLC, QbD, ICH, Azilsartan medoximil, Chlorthalidone.

INTRODUCTION

Azilsartan medoximil, chemically, (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}-1H-benzimidazole-7 carboxylate mono-potassium salt (fig. 1), is a new addition to the angiotensin receptor blocker (ARB) class of antihypertensive agents [1]. As an ARB, azilsartan medoxomil selectively inhibits angiotensin II from binding to the angiotensin II type-1 receptors (AT1) which causes the blocking of the pressor effects of angiotensin II and leads to antihypertensive activity [2, 3]. Azilsartan medoxomil is a type of prodrug. It gets hydrolysed to the active moiety, azilsartan, in the gastrointestinal (GI) tract during the absorption phase. The enzyme, principally responsible for the metabolism of azilsartan is cytochrome P450 (CYP) 2C9. Azilsartan is metabolized to two primary metabolites, M-I, and M-II, by decarboxylation and *O*-dealkylation, respectively. These metabolites have low affinity for the AT1 receptors and therefore, have no effect on the pharmacological activity of azilsartan medoxomil. Azilsartan medoximil is a white crystalline powder that is insoluble in water, freely soluble in methanol, soluble in acetic acid, slightly soluble in acetone and acetonitrile [4-6]. Chlorthalidone, chemically 2-chloro-5(1-hydroxy-3-oxo-1isoindolinyl) benzene sulphonamide (fig. 2), is a thiazide-like diuretic/antihypertensive. Chlorthalidone is a white or yellowish-white crystalline powder, practically insoluble in water, ether and chloroform, soluble in methanol and slightly soluble in ethanol. Chlorthalidone produces diuresis with increased excretion of sodium and chloride. The cortical diluting segment of the ascending limb of Henle's loop of the nephron is the site of action. The diuretic effects of chlorthalidone lead to the reduction in extracellular fluid volume, plasma volume, cardiac output, total exchangeable sodium, glomerular filtration rate, and renal plasma flow [3]. Variations in diuretic-mediated inhibition of carbonic anhydrase-dependent chloride transport in platelets and vascular smooth muscle could account for the contrasting efficacy of the thiazide and thiazide-like diuretics in reducing cardiovascular morbidity in patients with hypertension [7].

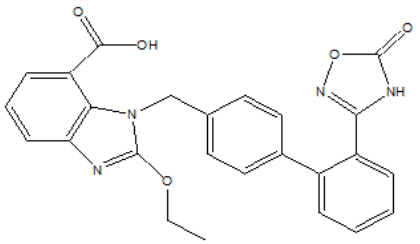

Fig. 1: Chemical structure of Azilsartan

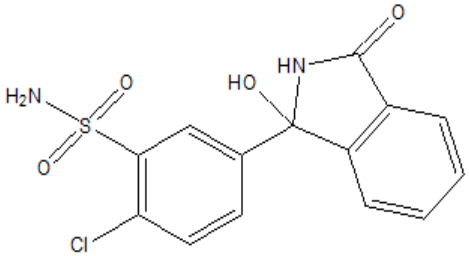

Fig. 2: Chemical structure of Chlorthalidone

The combination of azilsartan medoximil and chlorthalidone has

given promising results at low doses in a number of clinical trials carried out on the volunteers when compared with combinations of azilsartan medoximil and hydrochlorothiazide & other drug combinations of ARB such as olmesartan, ramipril, and irbesartan with chlorthalidone or hydrochlorothiazide [8-13]. During the literature survey, it was also found that combining chlorthalidone with other antihypertensive is more effective than hydro-chlorothiazide even in low doses [14]. This new combination of ARB (azilsartan medoximil) and diuretic (chlorthalidone) of brand name EDARBICHLOR, manufactured by Takada Pharmaceutical. U. S is very effective as an antihypertensive [15]. It was found to be more potent compared with other drug combinations or individual drugs [16]. Literature survey revealed that some analytical methods have already been reported for the determination of azilsartan medoximil alone or in combination with chlorthalidone like, spectrophotometry [17] and RP-HPLC [18, 19] method, but still there is scope to develop an easy, sensitive, specific, reliable and cost effective method. In the previous work C18 and ODS columns were used. However, it is reported in the literature that C8 column elutes the compounds in a shorter time and thus has shorter retention time than the C18 column with the same separation pattern as it has weaker hydrophobic interaction [20]. With the aim to optimize the RP-HPLC method with new conditions, the present work was undertaken using C8 column. The optimized method was validated according to the International Conference on Harmonisation (ICH) guidelines [24].

MATERIALS AND METHODS

Chemicals and reagents

Azilsartan medoximil and chlorthalidone were provided by reference standard division Indian Pharmacopoeia Commission (Ghaziabad, India) as gift samples. The drugs were characterized on the combined basis of the physical and instrumental analysis, i.e. IR, DSC, ^{1}HNMR and mass spectra. Acetonitrile (ACN), potassium hydroxide, sodium hydroxide, orthophosphoric acid 85 % were obtained from Merck (Mumbai, India) and potassium dihydrogen orthophosphate was purchased from HIMEDIA (Mumbai, India). Deionised Milli-Q water was obtained from Milipore (MA, USA) and hydrogen peroxide 85 % was obtained from Thomas baker (Mumbai, India). All the chemicals and reagents used were of analytical grade and HPLC grade.

Equipment

HPLC analysis was performed on a Thermo scientific Dionex ultimate 3000 UHPLC system integrated with binary gradient pump, autosampler and diode array detector. The output signal was monitored and integrated by using chameleon software.

Chromatographic conditions

The working conditions were selected after method development with different screening approaches**:** Acclaim TM 120, C8 (5 µm, 150×4.6 mm) enhanced polar selectivity column was used as stationary phase with mobile phase comprised of mixture of ACN: water (90:10) and potassium dihydrogen phosphate (KH_2PO_4) buffer solution (10 mmol) adjusted to the pH 2.8 with orthophosphoric acid in the ratio of 69.5:30.5. Injection volume was 10 µl and flow rate of the mobile phase was maintained at 0.8 ml/min and run time was 10 min. The column and sample temperatures were maintained at 20 ^oC and 5 ^oC respectively, and the effluent was set for detection at 220 nm.

Preparation of 10 mmol KH_2PO_4 buffer

Potassium dihydrogen phosphate (1.36 gm) was dissolved in milli-Q water to produce 1000 ml solution, shaken & sonicated for half an hour. It was filtered through 0.45µ filter paper.

Preparation of diluent

A mixture of ACN with water (90:10) and10 mmol KH_2PO_4 buffer in the ratio of (69.5:30.5) was shaken, sonicated and finally filtered through 0.45 µ filter paper.

Preparation of working standard solution

Azilsartan medoximil (50 mg) and chlorthalidone (50 mg) were transferred separately to two different volumetric flasks (50 ml), dissolved in diluent (10 ml), shaken, sonicated and final volume was made with diluent. It was again sonicated and filtered through 0.45 µ filter paper. It produced 1000 µg/ml solution of each drug as stock solutions. Azilsartan medoximil stock solution (4 ml) and chlorthalidone stock solution (1.25 ml) were transferred to a volumetric flask (100 ml) and volume was made using the diluent to get a concentration of 40 µg/ml of azilsartan medoximil and 12.5 µg/ml of chlorthalidone. The solution was sonicated and filtered through 0.45µ filter paper.

Preparation of sample solution of capsules containing azilsartan (40 mg) and chlorthalidone (12.5 mg)

Twenty capsules each containing ingredients as azilsartan medoximil (40 mg), chlorthalidone (12.5 mg) and excipients were weighed. The weight of 20 empty capsule shells was also recorded. The weight of the two drugs and excipients contained in a single capsule was determined, which was found to be 60.30 mg. This amount was transferred to a volumetric flask (100 ml) and dissolved in diluent (10 ml). The volume was made up to 50 ml and sonicated. The above solution (1 ml) was transferred to a volumetric flask (10 ml capacity), and volume was made using the diluent solution to get a concentration of 40 µg/ml of azilsartan medoximil and 12.5 µg/ml of chlorthalidone. This solution was sonicated and filtered through 0.45µ filter paper.

Method optimization

The identified method was optimized by quality by Design (QbD) as per the ICH Q8 guideline. The concept of QbD has been mentioned in the ICH Q8 guidelines, which state that "quality cannot be tested into products, but quality should be built in by design" [21]. The ideal QbD-based pharmaceutical development effort is accomplished through the use of multivariate experiments involving modern process controls enabling process understanding [22]. These parameters are analyzed, and a design space is generated. Understanding the design space for a pharmaceutical process generally involves the identification of critical attributes for the input materials, the process, and the final product [23]. For the optimization of the method, MINITAB software was used. MINITAB software has Central composite design (CCD) under the category of Response surface methodology (RSM) which was used to design a set of experimental runs by concerning the three independent variables viz. flow rate, buffer pH, and percentage of the buffer in the mobile phase. These three factors have an effect on the dependent variables viz. retention time, peak asymmetry, the number of theoretical plates and resolution. The final conditions optimized by the software include 0.8 ml/min. flow rate, 30.5 % of the buffer in the mobile phase with pH 2.8.

Method validation

The developed RP-HPLC method was validated to confirm that it was suitable for its intended purpose as described in ICH Q2 (R1) guidelines covering different parameters like specificity, linearity, accuracy, precision, robustness and system suitability [24]. ICH Q6A guidelines explicitly require forced decomposition studies to be conducted under a variety of stress conditions and separation of the pure drug from its degradation products for stability-indicating assay methods [25]. The described method was extensively validated in terms of linearity, accuracy, precision, specificity, system suitability, robustness, limits of detection and limit of quantification.

Linearity

Linearity was established from 50 % to 120 % of working standard concentration using minimum 8 calibration levels (50 %, 60 %, 70 %, 80 %, 90 %, 100 %,110 % and 120 %) having a range of 20 to 48 µg/ml for azilsartan medoximil and 6.3 to 15 µg/ml for chlorthalidone. The linearity of the method was evaluated by linear regression analysis. The calibration curve was plotted as the peak area of the working standard of substance against each concentration level.

Accuracy

The accuracy (recovery) of the method was determined on three

concentration levels (50 %, 100 % and 150 %) by the standard addition technique. The percentage recoveries of azilsartan medoximil and chlorthalidone at each level and at each replicate were determined. The mean of percentage recoveries (n = 9) and the relative standard deviation (RSD) was calculated.

Precision

Precision was evaluated with respect to repeatability (intraday and interday precision study). The intraday precision study was evaluated by analyzing working standard solution of both azilsartan medoximil and chlorthalidone at three concentration levels (80 %, 100 % and 120 %), on the same day. Similarly, the interday precision study was done with the same procedure as on day 1. The % RSD of the analytical responses was calculated.

Specificity (Forced degradation study)

Specificity studies were performed by exposing the bulk drug under different stress conditions. Azilsartan medoximil (40 µg/ml) and chlorthalidone (12.5 µg/ml) at which specificity studies were performed. The stress conditions used were:

a) 0.2 N HCL (Acidic study): Degradation studies were performed by subjecting the drug in 0.2 N HCL for 24 h.

b) 0.2 N NaOH (Alkaline study): Degradation studies were performed by subjecting the drug in 0.2 N NaOH for 24 h.

c) 5% H_2O_2: Degradation studies were performed by exposing the drug in 5% H_2O_2 for 24 h.

d) Thermal degradation: Degradation studies were performed by exposing the drug in the oven at 105 °C for 24 h.

Robustness

The robustness is a measure of method capacity to remain unaffected by small but deliberate changes in chromatographic conditions. This was studied by testing the influence of small changes in pH of the buffer (±0.2 units), organic (or buffer) content of mobile phase (±1.5 %) and flow rate (±1.5 %). The %RSD was calculated.

System suitability

System suitability parameters were measured so as to verify the system performance. System precision was determined on six replicate injections of working standard preparations (combination of azilsartan medoximil and chlorthalidone). All important characteristics, including the peak resolution, tailing, and theoretical plate number were measured.

Limits of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the method were obtained from equation (1) and (2)

$$LOD = 3.3*(\sigma/S) \ldots\ldots. (1)$$

$$LOQ = 10*(\sigma/S) \ldots\ldots (2)$$

Where, "σ" is the standard deviation of the intersection and "S" is the slope obtained from calibration curves of the linear study.

RESULTS AND DISCUSSION

Optimization

The method optimization was done using Minitab software version 16. Three factors two levels central composite design was used for condition optimization. The various independent factors optimized were flow rate, % of the buffer in the mobile phase and pH of the buffer. The limits of these variables were set to yield specific desired numerical conditions for retention time, theoretical plates, asymmetry (dependent variables). Fig. 3 shows the optimized conditions which could be used in the validation. Fig. 4 shows the chromatogram of the drugs at optimized conditions.

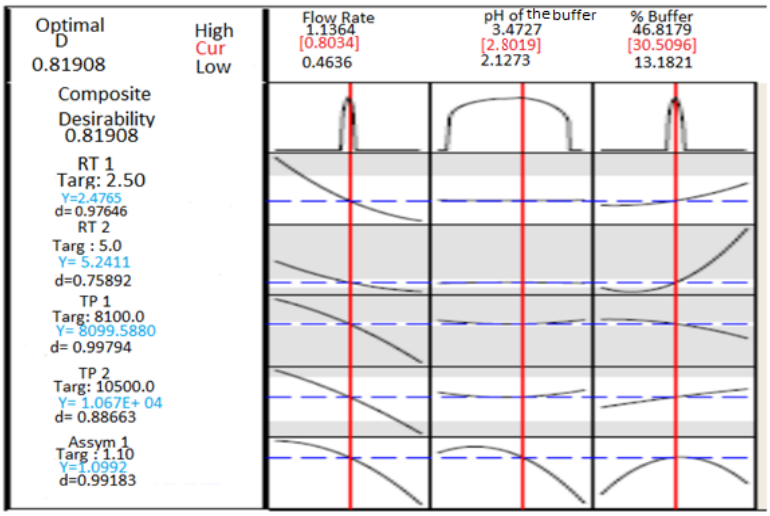

Fig. 3: Optimized conditions for the analysis of azilsartan medoximil and chlorthalidone

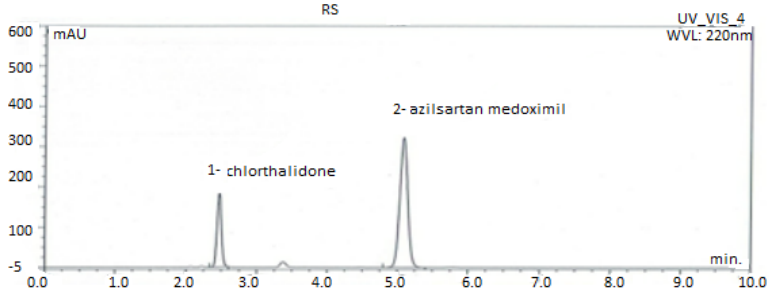

Fig. 4: Typical chromatogram of standard azilsartan medoximil and chlorthalidone

Validation of proposed method

Linearity

The response was found to be linear from 50 to 120 % of the working standard concentration (fig. 5, 6). The correlation coefficient for both drugs was 0.999. Correlation coefficients and linearity equations of the primary compounds are presented in table 1.

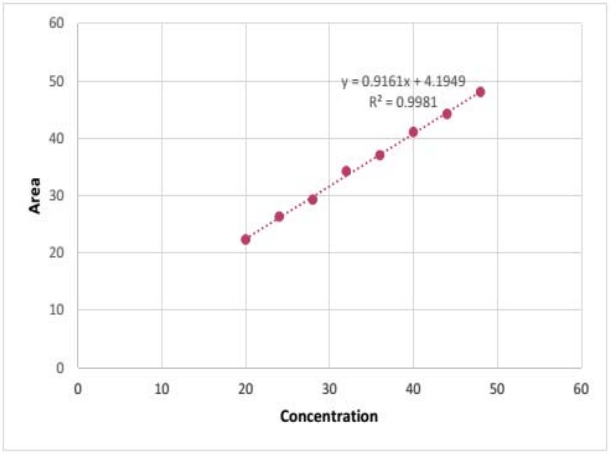

Fig. 5: Linearity plot of Azilsartan medoximil

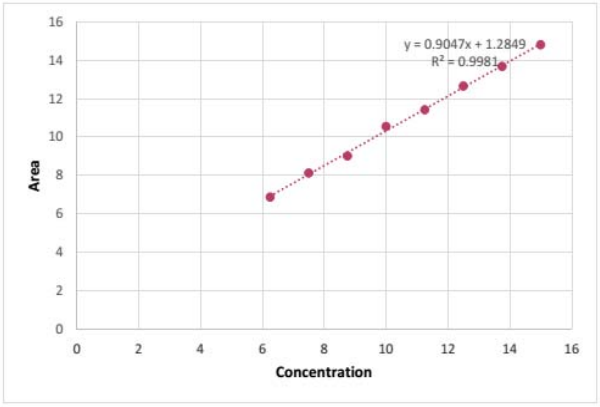

Fig. 6: Linearity plot of Chlorthalidone

Accuracy

The % recoveries were found within the range of 98.101 to 99.347 and 98.107 to 100.397 for azilsartan medoximil and chlorthalidone respectively. Thus, the amount recovered was within±2 % of the amount added, and the proposed method was found to be accurate. The results are summarized in table 2.

Precision

Precision study results are shown in table 3 and 4. The %RSD found to be <2% confirming the method to be sufficiently precise.

Table 1: Linearity regression data for azilsartan medoximil and chlorthalidone

Parameter	Azilsartan medoximil	Chlorthalidone
Linearity range	20 to 48 µg/ml	6.3 to 15 µg/ml
Regression equation	Y= 0.916X+4.1949	Y= 0.9047X+1.2849
Correlation coefficient (r^2)	0.999	0.999
Regression coefficient (R^2)	0.998	0.998
Slope	0.9161	0.9047
Intercept	4.1949	1.2849
LOD	1.537 µg/ml	0.472 µg/ml
LOQ	4.657 µg/ml	1.429 µg/ml

Table 2: Accuracy studies

%level	Azilsartan medoximil			Chlorthalidone		
	Recovery %	Mean recovery at each level ±SD	%RSD	Recovery %	Mean recovery at each level ±SD	%RSD
50	97.763	98.460±0.605	0.615	98.107	98.197±0.601	0.612
50	98.849			99.157		
50	98.769			99.136		
100	98.613	99.347±0.636	0.640	99.988	99.988±0.631	0.631
100	99.712			101.056		
100	99.715			101.104		
150	97.820	98.101±0.288	0.294	100.397	100.397±0.287	0.286
150	99.087			100.554		
150	98.397			100.954		
Mean recovery at each level ±SD	98.636±0.641			99.497±1.221		
%RSD	0.650			1.228		

SD= Standard Deviation, RSD= Relative Standard Deviation

Table 3: Precision studies for Azilsartan medoximil

Concentration level %	Repeatability			
	Intraday precision		Interday precision	
	Average area (mAU)a±SD	%RSD	Average area (mAU)b±SD	%RSD
80	34.350±0.121	0.352	33.684±0.033	0.099
100	40.762±0.123	0.301	40.350±0.407	1.088
150	50.367±0.105	0.209	49.648±0.094	0.188

SD= Standard Deviation, RSD= Relative Standard Deviation, a= Average of triplicate determinations in intraday precision, b= Average of triplicate determinations in interday precision.

Table 4: Precision studies for Chlorthalidone

Concentration level %	Repeatability			
	Intraday precision		Interday precision	
	Average area (mAU)a±SD	%RSD	Average area (mAU)b±SD	%RSD
80	10.694±0.030	0.285	10.485±0.008	0.074
100	12.891±0.099	0.148	12.566±0.009	0.069
150	16.049±0.020	0.125	15.884±0.031	0.193

SD= Standard Deviation, RSD= Relative Standard Deviation, a= Average of triplicate determinations in intraday precision, b= Average of triplicate determinations in interday precision.

Specificity (Forced degradation study)

The peaks of azilsartan medoximil and chlorthalidone were found to be pure except in alkaline studies, where azilsartan medoximil peak completely disappeared. Considerable degradation was observed during acidic (fig. 7), alkaline (fig. 8) and oxidative (fig. 9) conditions. A negligible amount of degradation had occurred in thermal condition (fig. 10), which was not considered relevant. During analysis, degraded peaks were observed in acidic, alkaline and oxidizing conditions. Moreover, other peaks in H_2O_2 and alkaline conditions were seen, which appeared in blank condition also, therefore, were not considered as the degradation peak.

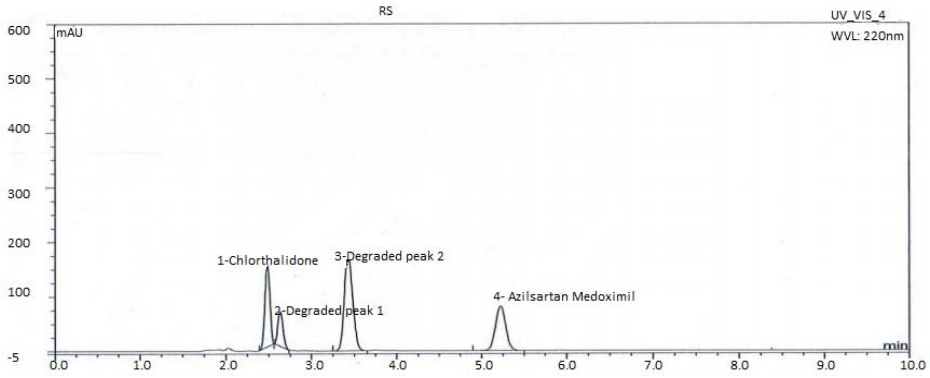

Fig. 7: Chromatogram of the acid degradation standard solution

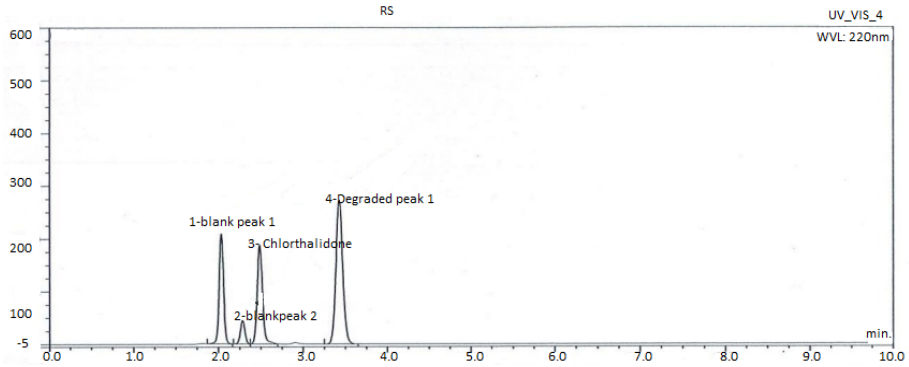

Fig. 8: Chromatogram of the alkaline degradation standard solution

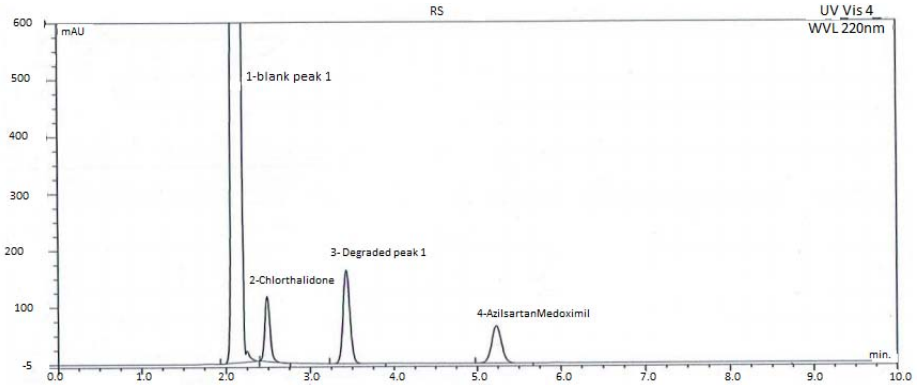

Fig. 9: Chromatogram of the oxidative degradation standard solution

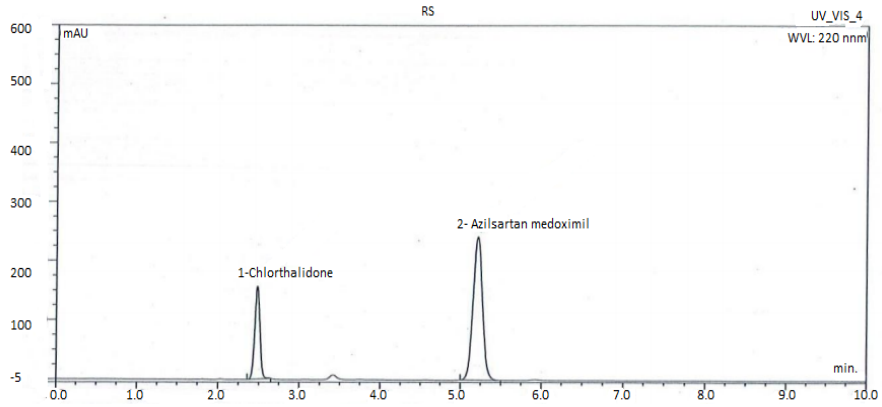

Fig. 10: Chromatogram of the thermal degradation standard solution

System suitability

The % RSD of the peak area of six replicated injection of working standard solutions of drugs was below 2.0 %, which shows that the system is precise. The results for the system suitability are presented in table 5.

Robustness

The % RSD for variation in flow rate, buffer content of mobile phase and pH of the buffer was found to be less than 2 % thus, confirming the method to be sufficiently robust. The result for robustness is presented in table 6.

Limits of detection and Limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) for azilsartan medoximil were 1.54 µg/ml and 4.66 µg/ml respectively and for chlorthalidone were 0.47 µg/ml and1.43µg/ml respectively.

Assay of capsule formulation

Assay of capsule formulation containing azilsartan (40 mg) and chlorthalidone (12.5 mg) was performed by using the above developed and validated RP-HPLC method. The potency of the formulation sample was found to be 108.116 % and 98.2 % respectively, which are under the acceptable limits as per I. P, shown in table 7.

Table 5: System suitability parameter

Parameters	Azilsartan medoximil	Chlorthalidone
Theoretical plates a	10066	7822
Tailing factor a	1.048	1.067
%RSD	0.213	0.178
Retention time a	5.09±0.5	2.47±0.5
Resolution	17	17

RSD= Relative Standard Deviation, a= Average of replicate determination

Table 6: Robustness studies of Azilsartan medoximil and Chlorthalidone

		Azilsartan medoximil			Chlorthalidone		
Parameter	**Variation**	**RT**	**Avg (%) ±SD**	**%RSD**	**RT**	**Avg (%) ±SD**	**%RSD**
Flow rate	0.812	5.10	5.17±0.081	1.562	2.45	2.48±0.035	1.414
	0.800	5.16			2.48		
	0.788	5.26			2.52		
% buffer in mobile phase (±1.5)	31	5.25	5.15±0.093	1.796	2.49	2.47±0.012	0.466
	30.5	5.15			2.47		
	30	5.07			2.47		
pH of buffer (±0.2)	3.1	5.15	5.15±0.003	0.056	2.48	2.47±0.006	0.233
	2.9	5.16			2.47		
	2.7	5.16			2.48		
Wavelength (±5)	225	5.16	5.16±0.0	0	2.48	2.48±0.0	0
	220	5.16			2.48		
	215	5.16			2.48		

SD= Standard Deviation, RSD= Relative Standard Deviation

Table 7: Results of the assay of capsule formulation containing Azilsartan medoximil and Chlorthalidone

S. No.	Formulation	Claim dose per capsule (mg)	Amount found (mg)a±SD	Potency &estimation (%)	% RSD
1.	Azilsartan medoximil	40	43.247±0.068	108.116	0.169
2.	Chlorthalidone	12.5	12.353±0.045	98.2	0.357

SD= Standard Deviation, RSD= Relative Standard Deviation, a= Average of triplicate determinations

CONCLUSION

An efficient, precise and rugged RP-HPLC method was successfully developed and validated for simultaneous determination of azilsartan medoximil and chlorthalidone in bulk form and capsule dosage form. The developed optimized method was validated as per ICH guidelines. All parameters were found to lie under the ICH guidelines. Method validation results have proven the method to be selective, precise, accurate, and robust, as well as stability indicating. This method can be successfully applied for the routine analysis, involving the determination of content uniformity and dissolution profiling as well as stability study by the industry. Drug assay in the formulation was performed by using this developed and validated method. The potency of the formulation was found to be 108.12 % and 98.20 % respectively, which are within the acceptable limits as per IP. It was found that the RP-HPLC method developed was better than previously developed RP-HPLC method due to its better resolution of 17, with low retention time of 2.4 and 5.1 min. for chlorthalidone and azilsartan medoximil respectively. The C8 column used for analysis also gave encouraging results with better resolution and less retention time. This method can be used in the routine analysis in QC laboratories. So we can save time, manpower and huge cost by using this method.

ACKNOWLEDGEMENT

We are thankful to Jamia Hamdard for the support, and facilities and also to the Indian Pharmacopoeia Commission, Govt. of India, Ministry of health and family welfare, Raj Nagar Ghaziabad (India) for providing samples of azilsartan medoximil and chlorthalidone, support and instrumental facilities.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/200796orig1s000chemr.pdf. [Last accessed on 12 Apr 2013].
2. Perry CM. Azilsartan medoxomil: a review of its use in hypertension. Clin Drug Invest 2012;32:621-39.
3. http://pharmacy.hsc.wvu.edu/media/1137/edarbyclor-azilsartan-chlorthalidone.pdf. [Last accessed on 15 Apr 2013].
4. Jones JD, Jackson SH, Agboton C, Martin TS. Azilsartan medoximil (edarbi). The Eighth Angiotensin II Receptor Blocker. P T 2011;36:634-6, 638-40.
5. Zaiken K, Cheng JW. Azilsartan medoxomil: a new angiotensin receptor blocker. Clin Ther 2011;33:1577–89.
6. Sica DA. Rationale for the use of fixed-dose combinations in the treatment of hypertension: the cycle repeats. Drugs 2002;62:2761–88.
7. Woodman R, Brown C, Lockette W. Chlorthalidone decreases platelet aggregation and vascular permeability and promotes angiogenesis. Hypertension 2010;56:463-70.
8. Bönner G, Bakris GL, Sica D. Comparison of antihypertensive efficacy of the new angiotensin receptor blocker azilsartan medoxomil with ramipril. J Hypertension 2010;28:283.
9. Weber MA, White WB, Sica D. Antihypertensive efficacy of the novel angiotensin receptor blocker azilsartan medoxomil in combination with amlodipine. J Hypertension 2010;28:279–80.
10. Bakris GL, Sica D, White WB, Cushman WC, Weber MA, Handley A, *et al*. Antihypertensive efficacy of hydrochlorothiazide vs chlorthalidone combined with azilsartan medoxomil. Am J Med 2012;125:1229.e1-1229.e10.
11. White WB, Weber MA, Sica D. Effects of the angiotensin receptor blocker azilsartan medoxomil versus olmesartan and valsartan on ambulatory and clinic blood pressure in patients with stages 1 and 2 hypertension. Hypertension 2011;57:413–20.
12. Sica D, Bakris GL, White WB. New angiotensin II receptor blocker azilsartan medoxomil coadministered with chlorthalidone provides potent blood pressure reduction in stage 2 hypertension. J Clin Hypertens 2010;12:A114.
13. Cushman WC, Bakris GL, White WB. Azilsartan medoxomil plus chlorthalidone reduces blood pressure more effectively than olmesartan plus hydrochlorthiazide in stage2 systolic hypertension. Hypertension 2012;60:310–8.
14. Ernst ME, Carter BL, Goerdt CJ. Comparative antihypertensive effects of hydrochlorothiazide and chlorthalidone on ambulatory and office blood pressure. Hypertension 2006;47:352-8.
15. http://www.takeda.us/newsroom/press_release_detail.aspx?id=231. [Last accessed on 07 Feb 2013].
16. Pierini D, Anderson KV. Azilsartan medoxomil/chlorthalidone: a new fixed-dose combination antihypertensive. Ann Pharmacother 2013;47:694-703.
17. Gorla R, Nagaraju CH, Sreenivasulu B, Sreenivas N, Korupolu RB. New simple UV Spectrophotometric method for determination of Azilsartan medoxomil in bulk and pharmaceutical dosage forms. Int J Res Pharm Biomed Sci 2013;4:1133-7.
18. Kasimala MB, Kasimala BB. Reverse phase-HPLC method development and validation for the simultaneous estimation of azilsartan medoxomil and chlortalidone in pharmaceutical dosage form. Jamonline 2012;2:117–26.
19. Sravani P, Rubesh S, Duganath N, Devanna N. Method development and validation for the simultaneous estimation of Azilsartan and Chlorthalidone by RP-HPLC in pharmaceutical dosage form. Indian J Pharm Sci 2014;4:725-9.
20. http://www.glsciences.com. [Last accessed on 16 Mar 2013].
21. Patil AS, Pethe AM. Quality by design (QbD): a new concept for development of quality pharmaceuticals. IJQPA 2013;4:13-9.
22. Vogt FG, Kord AS. Development of quality-by-design analytical methods. J Pharm Sci 2010;100:797-812.
23. Drenne JK. Quality by design–what does it really mean. J Pharm Innovation 2007;2:65-6.
24. International Conference on Harmonisation (ICH), Validation of analytical procedures: text and methodology Q2 (R1); 2005.
25. International Conference on Harmonisation (ICH),, Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances, Q6A; 1999.