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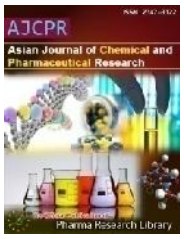

Research Article Open Access

Stability indicating RP-HPLC Method Development and Validation for the Simultaneous Estimation of Olmesertan Medoxomil and Chlorthalidone in Pharmaceutical Formulation

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A B S T R A C T

A new method was established for simultaneous estimation of Olmesertan medoxomol and Chlothalidone by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Olmesertan medoxomil and Chlorthalidone by using C18, 250 mm x 4.6 mm 5µ (Inertsil ODS), flow rate was 1.0ml/min, mobile phase ratio was Acetonitrile and water (70:30), detection wave length was 238nm. The instrument used was Shimadzu HPLC Auto Sampler, Separation module 2695, PDA Detector 2998, Empower-software version-2. The average retention times for Olmesartan Medoxomil and Chlorthalidone was found to be 3.5 and 6.7 min, respectively. According to United States pharmacopeia, system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 50µg/ml - 150µg/ml and 50µg/ml - 150µg/ml, with correlation coefficient 0.9999 and 0.9999 for Olmesartan Medoxomil and Chlorthalidone, respectively. The low values of RSD indicate that the method was precise and accurate. The effect of degradation products on the main peaks of Olmesartan Medoxomil and Chlorthalidone was determined by treating samples with different stress conditions like acid stress, alkali stress, peroxide stress, thermal stress and photolytic stress. The peak purity was found to be well below the purity threshold. Finally, it can be concluded that the assay values of formulation were the same as mentioned in the label claim with RSD of < 1.0%. The proposed method was found to be accurate, precise, reproducible and stable, and can be successfully applied for routine analysis of both the drugs in combined tablet dosage forms.

Keywords: Olmesertan medoxomil, Chlorthalidone, HPLC

A R T I C L E I N F O

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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 [1,2]. 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

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].

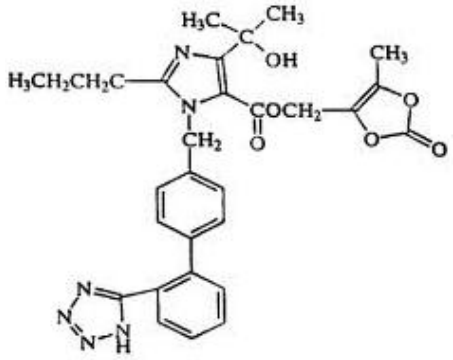


Figure 1: Olmesartan Medoxomil

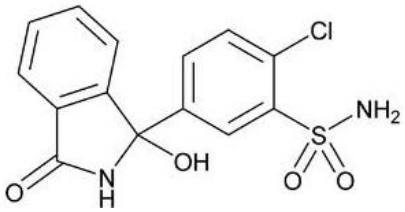


Figure 2: Chlorthalidone

2. Materials and Methods

Apparatus: Shimadzu Analytical Balance, Shimadzu UV-Spectrophotometer 1800 series with two matched quartz cells with a 1cm path length were employed in the method, Optics Technologies Ultrasonicator, Shimadzu HPLC.

Reagents and Materials:

The solvents used were Potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, Hydrogen peroxide, Acetonitril and Water [10].

Selection of detection wavelength:

From the standard stock solution III of Olmesartan Medoxomil and Chlorthalidone, appropriate aliquots were made with Acetonitrile and water (70:30) to obtain working standard solutions of concentrations from 1 to 50 µg / ml. Absorbance for these solutions were measured in the range of 200-400nm and the spectra was recorded[11].

Selection of mobile phase

Acetonitrile: Water (70: 30) pH was adjusted to 3.0 with Orthophosphoric acid. pH controls the elution properties by controlling the ionization characteristics. It also decreases the retention and improves separation [12]. Good Response, Tailing factor, Resolution will be achieved.

Optimization Chromatographic trials

Optimization chromatographic conditions

Column : C18, 250 mm x 4.6 mm 5µ (Inertsil ODS)
Mobile phase ratio : Acetonitrile : Water (70: 30)
Detection wavelength : 238nm
Flow rate : 1.0ml/min
Injection volume : 20µl Coumn Temperature

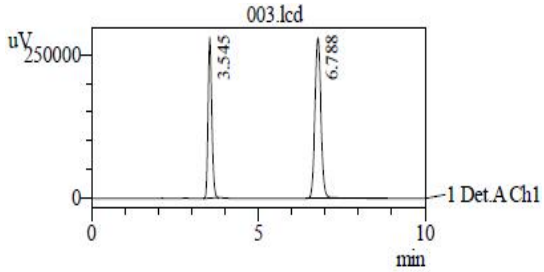


Figure 3: Optimization Chromatogram

Observation: The chromatogram is perfect with clear separation of components. The peak symmetry and system suitability parameters are within the limits. Hence this method is chosen as optimized one.

Procedure

Standard Preparation:

Weigh accurately and transfer about 40.0mg of Olmesartan Medoxomil working standard and 25.0mg of Chlorthalidone working standard into 100ml volumetric flask, add 70 ml of diluent, Sonicate with intermittent shaking to dissolve the contents and dilute to 100 ml with diluent and mix well. Further dilute 5ml of this solution to 25 ml volumetric flask and dilute to volume with diluents [13]. (The concentration of Olmesartan Medoxomil is about 80ppm and Chlorthalidone is about 50 ppm)

Preparation of sample solution:

Weigh and transfer 5 tablets into a 250 ml volumetric flask, then add about 140 ml of diluent, sonicated for about 20 min to dissolve the contents and dilute to 250 ml with diluent, mix well and filter through 0.45µm nylon filter. Further dilute 5 ml of this solution to 25ml volumetric flask and dilute to the volume with diluents (The concentration of Olmesartan Medoxomil is about 80ppm and Chlorthalidone is about 50 ppm) [14].

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 [15]. The specificity was performed by injecting blank [16].

2. Linearity

The linearity of an analytical method is its ability to elicit test results are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Individually, samples equivalent to 50%, 75%, 100%, 125%, 150% of the stated amount of sample were weighed individually and the assay was carried out as described earlier. A graph of weight taken versus chromatographic area ways plotted for both Olmesartan Medoxomil and Chlorthalidone peaks [16]. The regression line obtained was linear. From the data obtained, co-relation coefficient, slope, y-intercept were calculated. Ideally co-relation coefficient should be around.

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

3. Range:

The linearity study was performed for concentration range of 50µg-150µg and 50µg-150µg of Olmesartan medoxomil and Chlorthalidine and the correlation coefficient was found to be 0.999[17].

4. Accuracy

Accuracy of the method was determined by recovery experiments. There are mainly 2types of recovery studies are there [18].

Standard addition method:

To the formulation, the reference standard of the respective drug of known concentration was added, analyzed by HPLC and compared with the standard drug concentration.

Acceptance criteria:

The mean % recovery of the Olmesertan medoxomil and Chlorthalidine at each level should be not less than 95.0% and not more than 105.0%.

5. Precision

The precision of the method was demonstrated by intra-day and inter-day precision studies. Intra-day studies were performed by injecting three (3) repeated injections within a day. Peak area and %RSD were calculated and reported. The chromatograms of intra-day precision studies were shown. Inter-day precision studies, was done by injecting three (3) repeated injections for three consecutive days. Peak area and %RSD were calculated and reported [19].

Repeatability:

Repeatability was assessed by using a minimum of six determinations at 100% of the test concentration

Intermediate Precision

Intermediate Precision is demonstrated by carrying out the complete experiment by the different analyst on different days, on different instruments in the same laboratory [20].

Recovery studies

Sample solutions at different concentrations (50%, 75%, 100, 125 and 150%) were prepared and the % recovery was calculated [25].

Detection Limit:

The LOD was performed for Olmesartan Medoxomil and Chlorthalidone was found to be 1.964and 0.342 respectively.

Quantitation limit

The LOQ was performed for Olmesartan Medoxomil and Chlorthalidone was found to be 5.952 and 1.036 respectively.

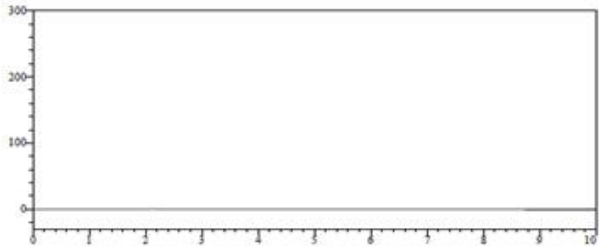


Figure 4: Chromatogram of Blank

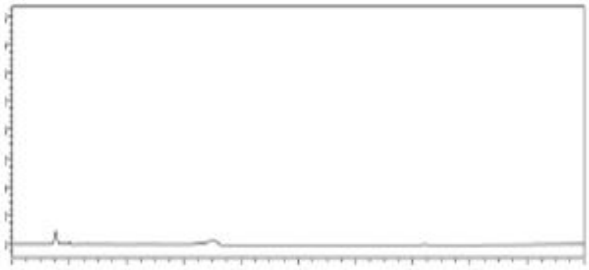


Figure 5: Chromatogram of Sample

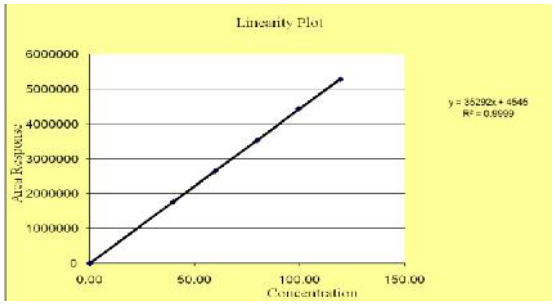


Figure 6: Calibration graph of Olmesartan medoxomil

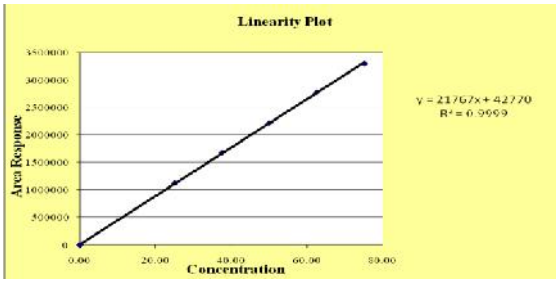
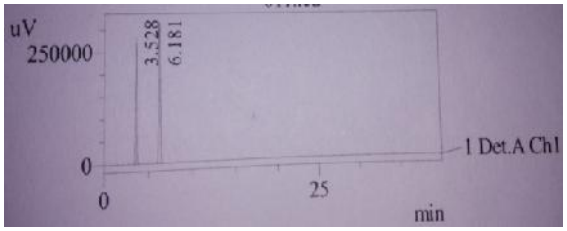
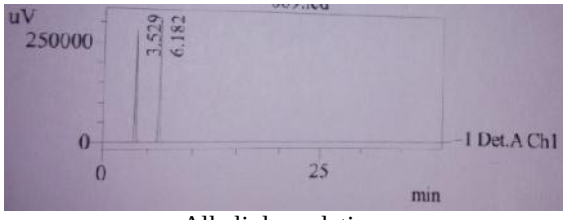


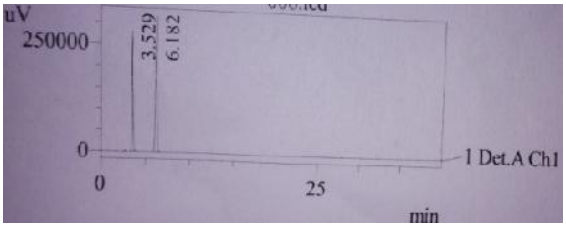
Figure 7: Calibration graph of Chlorthalidone



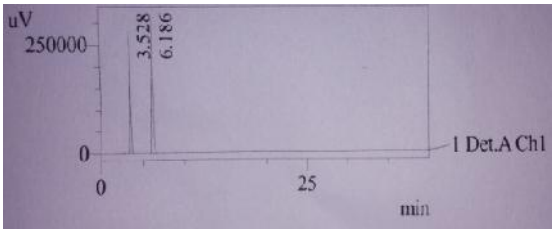
Acid degradation



Alkali degradation



Thermal degradation



Light degradation

4. Conclusion

The objective of the proposed work was method development for the simultaneous estimation of Olmesartan Medoxomil and Chlorthalidone in tablet dosage form by RP-HPLC and to validate the developed method according to USP and ICH guidelines and applying the same for use in the quality control samples in pharmaceutical industry. As there is no official method for simultaneous estimation of Olmesartan Medoxomil and Chlorthalidone in combination, so we tried to develop a method by which we can quantify the amount of drug present in the given sample. In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate titled ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with potassium dihydrogen phosphate and Acetone: Water (70:30) at Isocratic flow rate of 1.0ml min^{-1} was found to be quite robust. The optimum wavelength for detection was 238nm at which better response for both the drugs was obtained. The average retention times for Olmesartan Medoxomil and Chlorthalidone was found to be 3.5 and 6.7 min, respectively. According to United States pharmacopeia, system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 50µg/ml - 150µg/ml and 50µg/ml - 150µg/ml, with correlation coefficient 0.9999 and 0.9999 for Olmesartan Medoxomil and Chlorthalidone, respectively. The low values of RSD indicate that the method was precise and accurate. The mean recoveries were found in the range of 98 – 102 %. System precision is evaluated by injecting 10 replicate injections of standard solution and % RSD Shows that system is precise. Precision for method is evaluated by analyzing a sample of homogeneous batch six times and %RSD value shows the method is precise. Method robustness was evaluated by alteration of flow rate (±5%), Column temperature (±5%), and it was found robust as %RSD was below 2.0%. Both the sample solution and standard solution are stable at 25^0C for 24 hrs. As the % difference in the area was found to be less than 2.0%. Filter interference was done on three types 0.45µ filters (Nylon, PVDF and PDFE) and the %difference was found to be 2.0% for sample solution and standard solutions calculated against Centrifuged samples and standard.

The effect of degradation products on the main peaks of Olmesartan Medoxomil and Chlorthalidone was determined by treating samples with different stress conditions like acid stress, alkali stress, peroxide stress, thermal stress and photolytic stress. The peak purity was found to be well below the purity threshold. Finally, it can be concluded that the assay values of formulation were the same as mentioned in the label claim with RSD of < 1.0%. The proposed method was found to be accurate, precise, reproducible and stable, and can be successfully applied for routine analysis of both the drugs in combined tablet dosage forms.

Table 1: Calculation for Linearity of Olmesartan Medoxomil

Linearity Conc.	Area-Inj. 1	Area-Inj. 2	Avg. Response
50	1770146	1770162	1770154
75	2646082	2646228	2646155
100	3527407	3527731	3527569
125	4438431	4442284	4440357
150	5285706	5283442	5284574
Correlation Coefficient			**0.9999**
Slope(m)			**44270.8270**
Intercept (Y)			**4544.8000**
Statistical Y-Intercept			**0.1000**

Table 2: Calculation for Linearity of Chlorthalidone

Linearity Conc.	Area-Inj.1	Area-Inj.2	Avg. Response
50	1123622	1123909	1123765
75	1679903	1679259	1679581
100	2220302	2221305	2220803
125	2776272	2778749	2777510

150	3296220	3295122	3295671
Correlation Coefficient			**0.9999**
Slope(m)			**43490.9515**
Intercept(Y)			**42769.7012**
Statistical Y-Intercept			**1.9**

Table 3: Peak Results for Accuracy of Olmesartan Medoxomil

Target Conc.	Wt. taken	mg Spiked	Area Inj.1	Area Inj.2	Avg. Area	mg recovery	% recovery	Avg. Recovery	%RSD
50	50	49.9	1773959	1773065	1773512	50	100.2	100.2	0.0
50	50	49.9	1772508	1773177	1772843	50	100.2		
100	100	99.7	3548202	3547215	3547709	100.1	100.4	100.3	0.1
100	100	99.7	3539889	3545102	3542496	99.9	100.2		
150	150	149.6	5324164	5321355	5322760	150.1	100.3	100.3	0.0
150	150	149.6	5320095	5321871	5320983	150.1	100.3		
						Overall Recovery		**100.3**	**0.1**

Table 4: Peak Results for Accuracy of Chlorthalidone

Target Conc.	Wt. taken	mg Spiked	Area Inj.1	Area Inj.2	Avg. Area	mg recovery	% recovery	Avg. Recovery	%RSD
50	31.25	30.8	1124484	1124366	1124425	31.40	101.9		
50	31.25	30.8	1124042	1124297	1124170	31.40	101.9	101.9	0.0
100	62.50	61.6	2233036	2227205	2230121	62.20	101.0		
100	62.50	61.6	2232078	2230773	2231426	62.20	101.1	101.1	0.1
150	93.75	92.3	3316888	3314173	3315531	92.50	100.2		
150	93.75	92.3	3314677	3315246	3314962	92.50	100.2	100.2	0.0
						Overall Recovery		**101.1**	**0.1**

Table 5: Peak Results for Flow Changes of Olmesartan Medoxomil

Parameter	Flow plus (+5%)	RT	Flow Minus (-5%)	RT
Area (Inj.1)	3390503	6.412	3750161	7.092
Area (Inj.2)	3392732	6.412	3752692	7.094
Area (Inj.3)	3392827	6.411	3752291	7.093
Area (Inj.4)	3394565	6.411	3753218	7.092
Area (Inj.5)	3395619	6.410	3752613	7.093
Average Area & RT	3393249	6.411	3752195	7.093
%RSD	**0.058**		**0.032**	

Table 6: Peak Results for Flow Changes of Chlorthalidone

Parameter	Flow plus (+5%)	RT	Flow Minus (-5%)	RT
Area(Inj.1)	2130358	3.371	2364514	7.092
Area(Inj.2)	2130971	3.371	2364892	7.094
Area(Inj.3)	2131087	3.370	2364914	7.093
Area(Inj.4)	2131662	3.369	2364715	7.092
Area(Inj.5)	2132089	3.369	2364592	7.093
Average Area & RT	2131233	3.370	2364725	7.093
%RSD	**0.031**		**0.032**	

Table 7: Peak Results for System Precision

Olmesartan Medoxomil			Chlorthalidone		
Inj.	**RT (min)**	**Area(µV* sec)**	**Inj.**	**RT(min)**	**Area(µV* sec)**
1	6.788	3518913	1	3.545	2209696
2	6.778	3508955	2	3.547	2210386
3	6.773	3529870	3	3.547	2211199
4	6.772	3527342	4	3.546	2208192

5	6.774	3528731	5	3.547	2208959
6	6.771	3528896	6	3.546	2209003
7	6.770	3528938	7	3.547	2209589
8	6.767	3528753	8	3.545	2209267
9	6.768	3529016	9	3.547	2209458
10	6.768	3528838	10	3.547	2208925
Mean		3525825	**Mean**		2209467
%RSD		0.19	**%RSD**		0.038

Table 8: Peak Results for Method precision Precision

Olmesartan Medoxomil				**Chlorthalidone**			
Spl.no	**Inj.**	**RT(min)**	**Area**	**Spl.no**	**Inj.**	**RT(min)**	**Area**
Spl 1	1	6.765	3516291	Spl 1	1	3.547	2150510
	2	6.764	3517309		2	3.546	2152512
Spl 2	1	6.766	3514468	Spl 2	1	3.547	2151220
	2	6.765	3514117		2	3.546	2152091
Spl 3	1	6.763	3518905	Spl 3	1	3.545	2155676
	2	6.763	3516500		2	3.545	2154595
Spl 4	1	6.766	3516673	Spl 4	1	3.547	2154684
	2	6.765	3516153		2	3.547	2154403
Spl 5	1	6.767	3517204	Spl 5	1	3.546	2155411
	2	6.766	3517350		2	3.546	2155170
Spl 6	1	6.765	3526492	Spl 6	1	3.545	2161308
	2	6.764	3529139		2	3.545	2161410
Mean			3518383	**Mean**			2154916
%RSD			0.131	**%RSD**			0.160

Table 9: Degradation Conditions

Test Condition	**Acid stress**	**Alkali stress**	**Peroxide stress**	**Heat stress**	**Photolytic stress**
Olmesartan Medoxomil	3N HCl, 80˚C,2hr	1N NaOH	6%, 5min	80˚C 48hrs	1.2million lux hrs
Chlorthalidone	3N HCl, 80˚C, 2 hr	1N NaOH	6%, 5min	80˚C 48hrs	1.2million lux hrs

Table 10: Results of Forced Degradation Studies for Olmesartan Medoxomil

Test No	**Unstressed**	**Acid stress**	**Alkali stress**	**Peroxide stress**	**Heat stress**	**Photolytic stress**
Average weight	**155.5**					
Wt taken (in mg)	**774.3**	**774.2**	**775.5**	**774.9**	**775.1**	**775.4**
Area (Injection 1)	3516291	2927637	3072376	2812506	3507836	3496338
Average Area	3516291	2927637	3072376	2812506	3507836	3496338
Assay (mg/Tab)	**19.92**	**16.58**	**17.37**	**15.92**	**19.85**	**19.77**
Assay (in %)	**99.4**	**82.9**	**86.9**	**79.6**	**99.2**	**98.9**
% Degradation	**NA**	**16.8**	**12.8**	**20.1**	**0.3**	**0.7**

Table 11: Results of Forced Degradation Studies for Chlorthalidone

Test No	**Unstressed**	**Acid stress**	**Alkali stress**	**Peroxide stress**	**Heat stress**	**Photolytic stress**
Average weight	**155.5**					
Wt taken (in mg)	**774.3**	**774.2**	**775.5**	**774.9**	**775.1**	**775.4**
Area (Injection 1)	2150510	2131801	2128810	2124435	2125879	2137278
Average Area	2150510	2131801	2128810	2124435	2125879	2137278
Assay (mg/Tab)	**12.25**	**12.15**	**12.11**	**12.09**	**12.10**	**12.16**
Assay (in %)	**98.0**	**97.2**	**96.9**	**96.7**	**96.8**	**97.3**
% Degradation	**NA**	**0.8**	**1.1**	**1.3**	**1.2**	**0.7**

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