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METHOD DEVELOPMENT AND VALIDATION OF RELATED IMPURITIES FOR ASSAY OF OLMESARTAN MEDOXOMIL AND HYDROCHLORTHIAZIDE IN SOLID ORAL TABLETS BY RP-HPLC

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ABSTRACT

The purpose of this work is to develop an accurate and precise HPLC method for the determination of Hydrochlorothiazide and Olmesartan Medoxomil in solid dosage form. Separation of the drug was achieved on an inertsil ODS 3V 150x4.5 column using a mobile phase consisting of 0.01M pH 3.0 phosphate buffer and acetonitrile by using a gradient programmer. The flow rate was 1.2ml/min and the detection wavelength was 262nm. The linearity was observed in the range of 15-90p-pm for Hydrochlorothiazide and 24-144ppm for Olmesartan Medoxomil with a correlation coefficient of 0.999 and 0.999 respectively. The impurities of Hydrochlorthiazide and Olmesartan Medoxomil were optimized and separated. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine control analysis of Hydrochlorothiazide and Olmesartan Medoxomil in solid dosage form by RP-HPLC.

Keywords: Hydrochlorothiazide, Olmesartan Medoxomil, impurity profiling, dosage form RP-HPLC, validation

INTRODUCTION

Olmesartan Medoxomil is chemically 4-(1-hydroxy-1-methylethyl)-2-propyl-1-{[2'-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl}-1H-imidazole-5-carboxylic acid. Its molecular formula is $C_{24}H_{26}N_6O_3$ and its molecular weight is about 446.51g/mol. Olmesartan belongs to Angiotensin II receptor blocker which selectively binds to the Angiotensin receptor 1(AT1) and prevent the protein Angiotensin II from binding thereby leads to reduced blood pressure, lower aldosterone levels, reduced cardiac activity and increased excretion of sodium. Olmesartan is commonly used for the management of hypertension and Type-2 diabetes associated with nephropathy, particularly in patients who are unable to tolerate ACE inhibitors. Olmesartan when administered orally, bioavailability increases from 4.6% to 28.9%, it is 99% highly bounded to plasma proteins and does not penetrate into RBC. The mean plasma Olmesartan half-life is about 10-15hours and eliminated through feces.

Hydrochlorthiazide is chemically 6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1λ^6,2,4-benzothiadiazine-7-sulfonamide. Its molecular formula is $C_7H_8ClN_3O_4S_2$ and molecular weight is about 297.74g/mol. Hydrochlorthiazide is the most commonly used thiazide diuretic to treat edema associated with congestive heart failure, chronic renal failure, acute glomerulonephritis and nephritic syndrome. Hydrochlorthiazide acts on the proximal region of the distal convoluted tubule inhibiting the reabsorption by sodium chloride symporter, thereby reduces the concentration of sodium ions between the epithelial cells and distal convoluted tubule, decreasing the reabsorption of water. It is 40-68% bounded to plasma proteins, eliminated through urine and its plasma half-life is about 15hours.

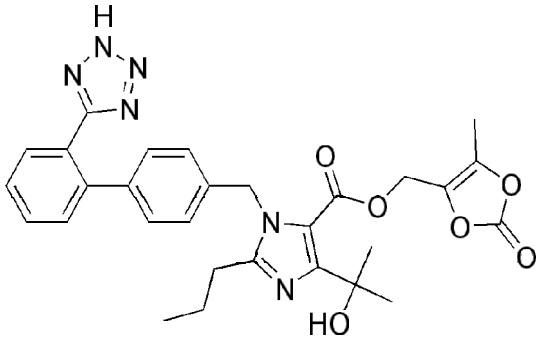

Figure 1: Olmesartan Medoxomil

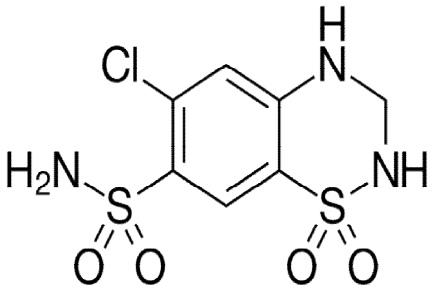

Figure 2: Hydrochlorthiazide

IMPURITIES

Olmesartan Medoxomil impurities As per USP,

1. Olmesartan acid impurity
2. Ethyl Ester Olmesartan Impurity
3. Dehydro Olmesartan Impurity
4. Trityl Alcohol Olmesartan Impurity

Hydrochlorthiazide impurities as per USP

1. Benzothiadiazine Related Compound A
2. Chlorthiazide Impurity
3. 5-Chlor Hydrochlorthiazide Impurity

MATERIALS AND METHODS

Chromatographic conditions

Chromatographic conditions were achieved using Inertsil C18, (150×4.6mm, particle size 5µ) column for separation. Flow rate of 1.2mL/min. Wavelength at 252nm with an injection volume of 20µL and run time was about 18 minutes at temperature of 30^0C.

Preparation of mobile phase (pH 3.0 phosphate buffer):

1.36gm of potassium dihydrogen phosphate (KH_2PO_4) in 1000ml water and adjust pH to 3.0 ± 0.05 with diluted ortho-phosphoric acid solution.

Preparation of diluent:

Mix Acetonitrile, Methanol and Water in the ratio of 50:20:30 and keep it for sonication to degas the solution.

Preparation of standard solution:

Weigh accurately 80mg of Olmesartan and 50mg of Hydrochlorothiazide working standard into a 100ml volumetric flask, add 80ml of diluent and keep it for sonication and diluent to volume with diluent. Pipette out 3ml of the above standard stock solution into a 25 ml volumetric flask, dilute to volume with diluent. Filter the solution through 0.45µm PVDF syringe filter.

Preparation of test solution:

Weigh and transfer 10 tablets into 250ml volumetric flask and add 10ml of water and shake the flask to disintegrate the tablets. Add 200ml of diluent sonicate for about 25mins with intermittent shaking, dilute to volume with diluent. Filter the above solution with 0.45 micron PVDF filter. Pipette 3 ml of the above solution into a 50ml volumetric flask and dilute to volume with diluent.

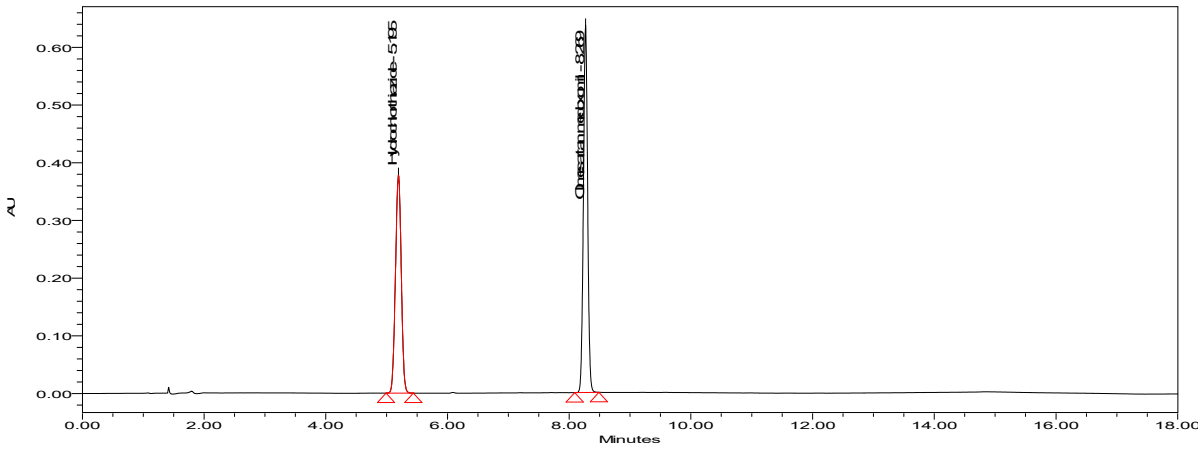

Figure 3: Standard chromatogram

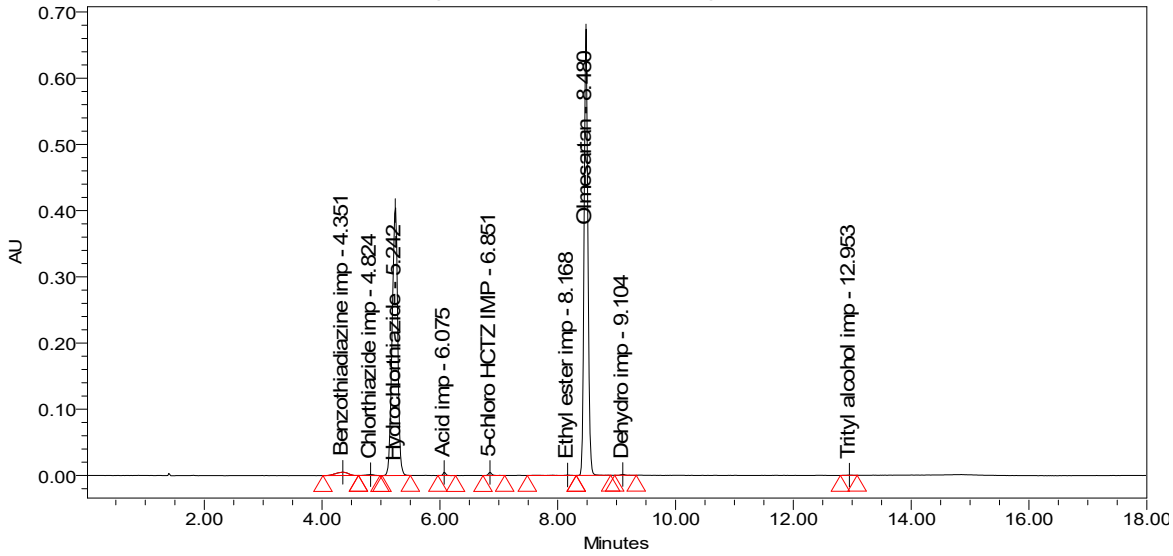

Figure 4: Chromatogram of sample preparation spiked with known impurities

Table: 1 Results of Impurities interference

S.No	Name	Retention Time	Purity Angle	Purity1 Threshold	Area	USP resolution	USP tailing	USP Plate count
1	Benzothiadiazine imp	4.351	-	-	75170		0.9	-
2	Chlorthiazide imp	4.824	-	-	13912	1.6	0.9	-
3	HCTZ	5.242	0.162	0.315	2661789	2.2	0.9	14091
4	5-chlor HCTZ imp	6.851	-	-	19706	6.2	1.1	-
5	Acid imp	6.075	-	-	16351	8.1	1.3	-
6	Ethyl ester imp	8.168	-	-	11641	9.2	0.6	-
7	OLMESARTAN	8.480	0.141	0.396	3009856	2.1	1.1	80536
8	Dehydro imp	9.104	-	-	7225	4.9	1.4	-
9	Trityl alcohol imp	12.953	-	-	2936	23.4	0.8	-

METHOD VALIDATION

SYSTEM SUITABILITY

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time, number of theoretical plates and tailing factor were evaluated for six replicate injections of the drug at a concentration of 96 µg/ml, 60µg/ml.

Acceptance criteria

The % RSD should be NMT 2.0%, the number of theoretical plates (N) should be NLT 2000 and the Tailing factor (T) should be NMT 2.0

SPECIFICITY

Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. Equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Acceptance criteria

The chromatogram of placebo should not show any peak at the retention time of the main analyte. The retention time should be identical for both the standard and sample chromatograms.

LINEARITY

Prepare a series of standard solutions (not less than 5 is recommended) in the range of 15µg/ml-75 µg/ml of Hydrochlorthiazide standard and 24µg/ml-144µg/ml of Olmesartan Medoxomil standard injected. A plot of average peak area versus the concentration in µg/ml or mg/ml is made and from this the correlation coefficient, y-intercept (const. of regression) and slope (coefficient of regression) of the regression line were calculated.

Acceptance criteria: The correlation coefficient should not be less than 0.999

PRECISION

The precision of the test procedure was evaluated for Hydrochlorthiazide and Olmesartan Medoxomil by injecting the six standard solutions. The Relative Standard Deviation of six injections was calculated.

Acceptance criteria

The % RSD should be NMT 2.0%, the number of theoretical plates (N) should be NLT 2000 and the Tailing factor (T) should be NMT 2.0

ACCURACY

To validate the test method can accurately quantify Hydrochlorthiazide and Olmesartan Medoxomil, prepare samples in three times for higher and lower levels, in triplicate for other levels by spiking Hydrochlorthiazide and Olmesartan Medoxomil, active material

with equivalent amount of placebo and perform CU as per test procedure. Prepare samples at levels 50%, 100% and 150% of the target assay concentration i.e. 50% of the lowest strength initial concentration to 150% of the highest strength initial concentration level.

Acceptance criteria

The mean % recovery for each spiked level should be not less than 98% and not more than 102%.

ROBUSTNESS:

Robustness of the method is performed by altering the chromatographic conditions such as changing the flow rate, change of temperature, Mobile phase composition and observed the variation of the results which should be within the acceptance criteria.

LIMIT OF DETECTION (LOD):

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

1. Based on Signal-to-Noise for LOD (3:1), LOQ (10:1)
2. Based on the Standard Deviation of the Response and the Slope

LIMIT OF QUANTIFICATION (LOQ):

The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

From the linearity data, the limit of detection and quantification were calculated using the following formula.

$$\mathbf{LOD} = \frac{3.3\,\sigma}{S}, \quad \mathbf{LOQ} = \frac{10\,\sigma}{S}$$

σ = standard deviation of the response, S = slope of the calibration curve

LOD and LOQ of Hydrochlorthiazide and Olmesartan are performed by spiking of known concentrations of the sample into the placebo of formulation and inject the sample.

RESULTS AND DISCUSSION

SYSTEM SUITABILITY

Table: 2 Results from system suitability studies of Hydrochlorthiazide and Olmesartan Medoxomil

System suitability parameters	Observed value		Acceptance criteria
	Hydrochlorthiazide	Olmesartan Medoxomil	
The Tailing for Hydrochlorthiazide and Olmesartan Medoxomil in standard solution	1.0	1.0	≤ 1
Theoretical plates for Hydrochlorthiazide and Olmesartan Medoxomil in standard solution	140140	68903	NLT 5000
% Relative standard deviation for Hydrochlorthiazide and Olmesartan Medoxomil peak area from six replicate injections of standard solution	0.5	0.2	≤ 2

SPECIFICITY

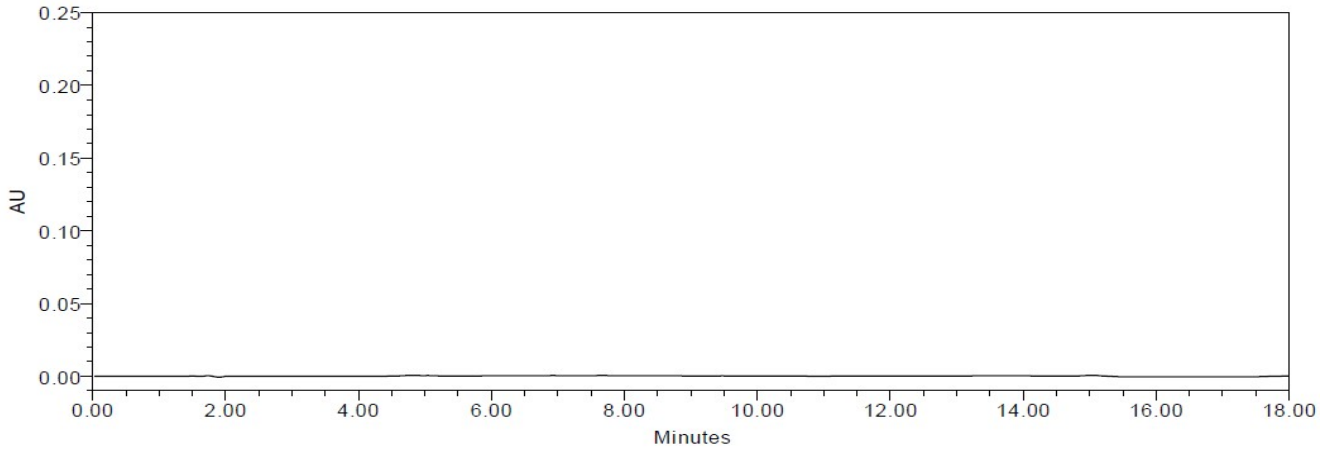

Figure 5: Chromatogram of Placebo solution

LINEARITY

Table: 3 Results of Linearity of detector response

Standard concentration(µg/ml)		Area		Mean Area	
HCTZ	OLM	HCTZ	OLM	HCTZ	OLM
15	24	655529	779478	655490	779465
		655452	779452		
30	48	1301829	1533483	1308178.5	1531469
		1314528	1529456		
45	72	1957196	2301075	1951546	2305763
		1945896	2310452		
60	96	2558901	3014981	2558558	3014618
		2558215	3014256		
75	120	3223312	3770802	3223929	3768107
		3224547	3765412		
90	144	3810231	4480284	3810046	4480262
		3809862	4480241		
Regression Hydrochlorthiazide=0.999 Olmesartan Medoxomil=0.999					

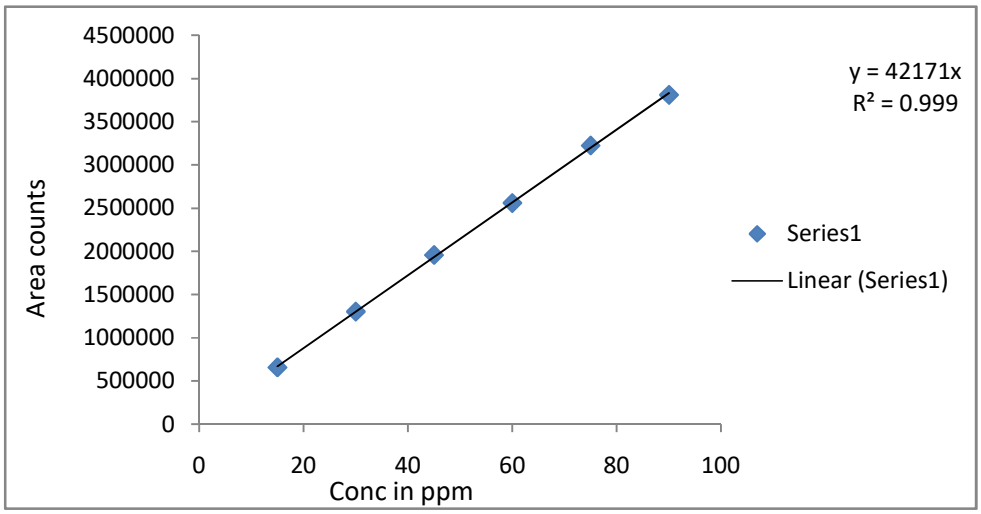

Fig.6: Linearity of Hydrochlorthiazide

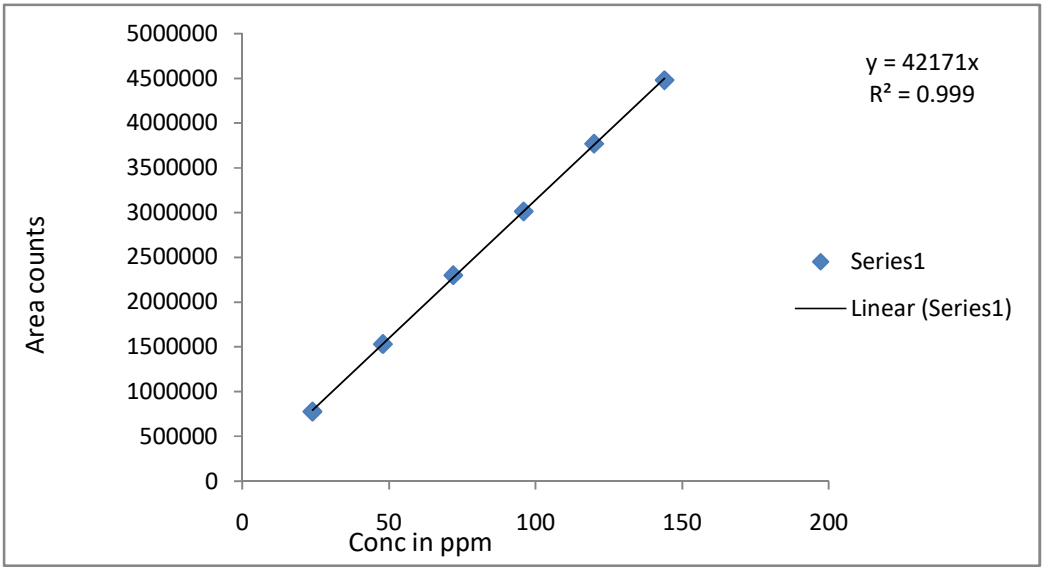

Fig.7: Linearity of Olmesartan Medoxomil

PRECISION

Table 4: Precision for Hydrochlorthiazide and Olmesartan

S.NO	Injection Number	Peak area for Hydrochlorthiazide	Peak area for Olmesartan Medoxomil	Acceptance Criteria
1	Standard 1	2540913	3095658	The %RSD for peak area of Hydrochlorthiazide and Olmesartan Medoxomil from six replicate injections of standard solution should not be more than 2.0
2	Standard 2	2509496	3091455	
3	Standard 3	2504314	3084434	
4	Standard 4	2507286	3086961	
5	Standard 5	2512208	3091031	
6	Standard 6	2521153	3096891	
Mean		2515895	3091072	
%RSD		0.5	0.2	

ACCURACY

Table 5: Results of accuracy of Hydrochlorothiazide

%Level spiked	Sample No.	% Recovery	Mean % Recovery	% RSD
50	1	100.8	99.6	1.06
	2	98.8		
	3	99.2		
100	1	100.4	99.7	0.6
	2	99.2		
	3	99.6		
150	1	98.4	99.6	1.57
	2	99.5		
	3	101.5		

Table 6: Results of accuracy of Olmesartan

% Level spiked	Sample No.	% Recovery	Mean % Recovery	% RSD
50	1	99.8	99.5	0.2
	2	99.5		
	3	99.3		
100	1	99.8	99.7	0.2
	2	99.5		
	3	99.8		
150	2	100.2	100.2	0.6
	3	99.7		
	2	100.8		

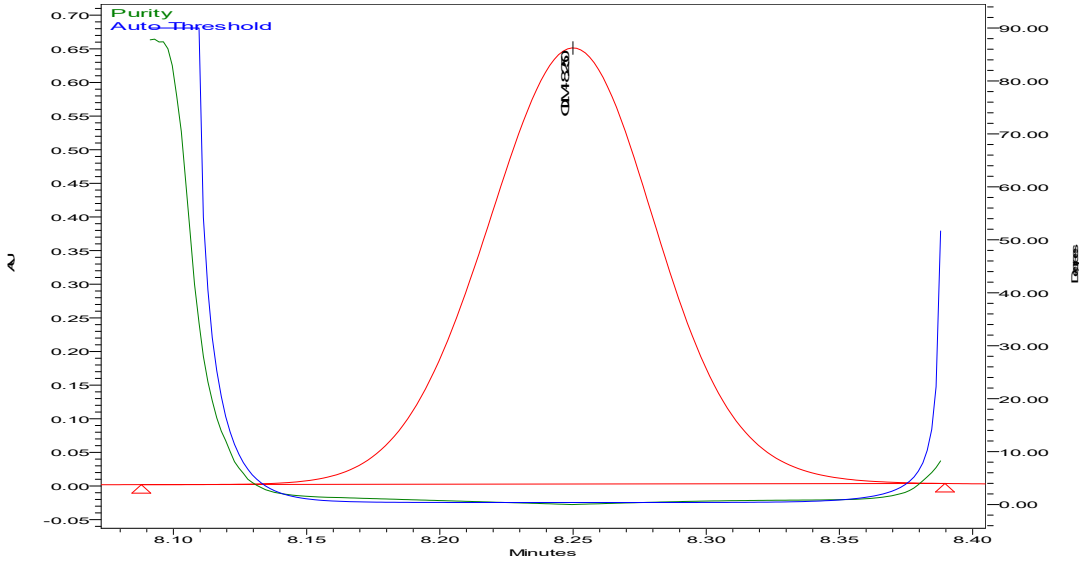

Fig: 8 Purity of Hydrochlorthiazide in sample preparation spiked with known impurities

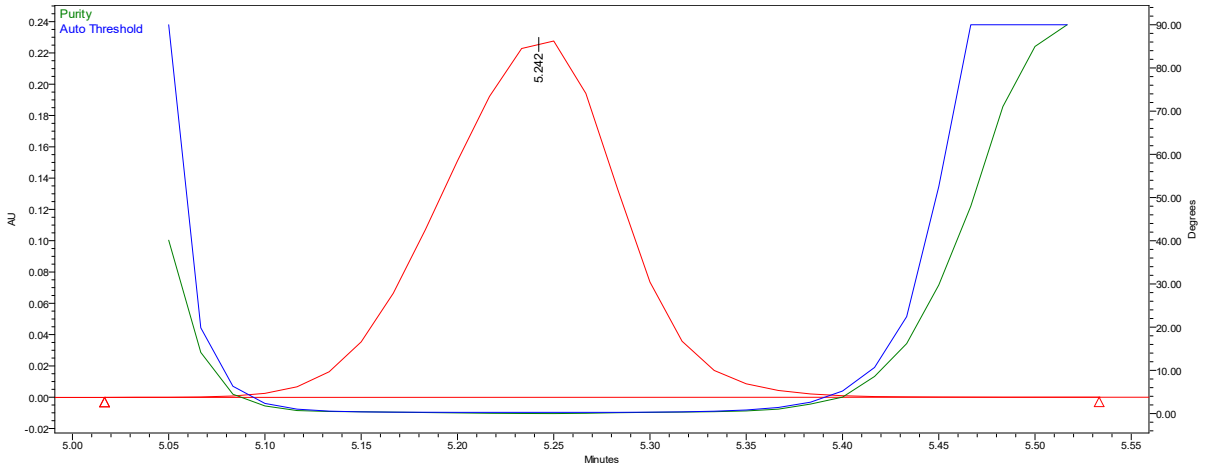

Fig: 9 Purity plot of Olmesartan Medoxomil in sample preparation spiked with known impurities

ROBUSTNESS

Effect of variation in Flow rate:

A study was conducted to determine the effect of variation in flow rate. The system suitability parameters were found to be within the limits for higher and lower flow rates of 1.3 ml/min. and 1.1 ml/min **(Table 7)**.

Effect of variation in column oven temperature:

A study was conducted to determine the effect of variation in column oven temperature. The system suitability parameters were evaluated at 25°C and 35°C column oven temperatures. The system suitability results were found to be within the limits at both column oven temperatures.

Table 7: Results of Robustness – Hydrochlorthiazide

System suitability criterion of Hydrochlorthiazide	Observed value at flow rate		Acceptance criteria
	1.1 ml/min	1.3 ml/min	
The USP Plate count	13130	9184.64	MT 2000
The Tailing factor from the chromatogram of Standard solution	0.9	1.04	≤ 1.0
% Relative standard deviation for five replicate injections of standard solution	0.8	0.2	≤ 2.0

Table 8: Results of Robustness – Olmesartan Medoxomil

System suitability criterion of Olmesartan	Observed value at flow rate		Acceptance criteria
	1.1 ml/min	1.3 ml/min	
The USP Plate count	79762	75369	MT 2000
The Tailing factor from the chromatogram of Standard solution	1.0	1.0	≤ 1.0
% Relative standard deviation from five replicate injections of standard solution	0.8	0.2	≤ 2.0

Table 9: Results of Robustness -Hydrochlorthiazide (effect of variation in column oven temperature)

System suitability criterion Hydrochlorthiazide	Observed value at column oven temp.		Acceptance criteria
	25°C	35°C.	
The USP Plate count	18459	13452	MT 2000
The Tailing factor from the chromatogram of Standard solution	0.9	0.9	≤ 1.0
% Relative standard deviation from five replicate injections of standard solution	0.1	0..2	≤ 2.0

Table 10: Results of Robustness –Olmesartan Medoxomil (effect of variation in column oven temperature)

System suitability criterion Olmesartan	Observed value at column oven temp.		Acceptance criteria
	25°C	35°C.	
The USP Plate count	25231	27444	MT 2000
The Tailing factor from the chromatogram of Standard solution	0.93	0.95	≤ 1.0
% Relative standard deviation from five replicate injections of standard solution	0.3	0.2	≤ 2.0

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:

Table 11: Results of LOD and LOQ

Sample	LOD	LOQ
Hydrochlorthiazide	0.1208 µg/ml	0.3661µg/ml
Olmesartan	1.3737 µg/ml	4.1627 µg/ml

DISCUSSION

A simple, sensitive, stability indicating and precise HPLC method for estimation of Olmesartan Medoxomil (40mg) and Hydrochlorthiazide (25mg) tablets has been developed and validated for determination in commercial dosage forms. The compound and its impurities were well separated by using gradient programme on a C18 column (inertsil ODS 3V, 5µ, 150*4.6mm) using a mobile phase consisting of pH 3.0 phosphate buffer: Acetonitrile at a flow rate of 1.2

ml/min with UV detection at 262nm. The retention time of Hydrochlorthiazide peak was at 5.242 and Olmesartan peak was at 8.480. The procedure was validated for all compendial and non compendial parameters in accordance with ICH guidelines. The study showed that the reverse phased liquid chromatography is sensitive and selective for detecting Hydrochlorothiazide, Olmesartan and its impurities using the gradient programme. Hence, the chromatographic method developed for Olmesartan Medoxomil and Hydrochlorothiazide is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

CONCLUSION

The HPLC method was found to be accurate, precise and reproducible. The method can be applied for routine estimation of Olmesartan Medoxomil (40mg) and Hydrochlorthiazide (25mg) in pharmaceutical formulations. The method was validated by using various validation parameters like system suitability, specificity, linearity, precision, accuracy, solution stability, filter interference and robustness. All the validation parameters were found to be within the acceptance criteria. Therefore, the proposed method can be used for routine analysis for the estimation of Olmesartan Medoxomil and Hydrochlorthiazide in its tablets formulation.

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